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Review Article

Spatial Tumor Heterogeneity: The Next Frontier in Understanding Cancer Resistance

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Abstract:

Spatial tumour heterogeneity, which denotes the changes in cellular and molecular attributes across distinct locations within a tumour, significantly influences cancer diagnosis and treatment resistance. The heterogeneity of tumour cells inside a singular mass facilitates tumour development, metastasis, and the ineffectiveness of standard therapy. Comprehending the geographical distribution of tumour cells is crucial for formulating more efficient treatment regimens. Diverse methodologies are employed to investigate spatial heterogeneity, encompassing modern imaging techniques such as MRI, PET, and multiplexed imaging, alongside omics approaches including genomes, transcriptomics, and proteomics. These instruments offer insights into the tumour microenvironment and facilitate the identification of resistant subpopulations. The amalgamation of imaging and genomic data via radiogenomics has emerged as a viable methodology, providing an extensive perspective on the spatial and molecular intricacies of tumours. Principal findings reveal that spatial heterogeneity fosters medication resistance by establishing microenvironments characterised by varying oxygen levels, immunological infiltration, and genetic alterations, hence complicating the efficacy of monotherapy strategies. Hypoxic environments and immunological evasion significantly contribute to treatment resistance. Addressing geographical heterogeneity has the potential to enhance cancer treatments. By analysing the molecular and geographical characteristics of tumours, physicians can customise therapies more efficiently, minimising resistance and improving therapeutic results. This methodology signifies a vital advancement in precision medicine, providing more individualised and efficacious cancer therapies in the future.

Keywords: Spatial Tumor Heterogeneity; Cancer Resistance; Imaging Techniques; Radiogenomics; Drug Resistance; Precision Medicine; Tumor Microenvironment; Hypoxia; Immune Evasion; Multiplexed Imaging; Genomics; Proteomics

Introduction

Cancer heterogeneity is a defining characteristic of tumour biology, significantly impacting cancer diagnosis, prognosis, and treatment. Tumour heterogeneity denotes the existence of genetic and phenotypic variation within an individual tumour, significantly influencing the progression, metastasis, and therapeutic resistance of cancers. This complexity can be classified into two primary categories: genetic and geographic heterogeneity [1]. Genetic heterogeneity denotes the variations in the genetic composition of tumour cells, frequently influenced by mutations, amplifications, and deletions. Spatial heterogeneity denotes the variability in tumour features throughout distinct parts of the tumour microenvironment (TME) and is increasingly acknowledged for its significant influence on cancer biology [2]. The spatial organisation of cancer cells within the tumour is regulated by genetic variables as well as microenvironmental elements, including hypoxia, nutrition gradients, and immune cell infiltration. This spatial heterogeneity represents a novel frontier in cancer research, as it affects tumour behaviour and therapy response in ways that are just starting to be comprehended [3].

Spatial heterogeneity inside tumours is becoming recognised as a vital element in cancer treatment. It denotes the heterogeneous dispersion of tumour cells within a singular tumour mass, characterised by different microenvironments that differ in molecular composition, metabolic status, and treatment susceptibility [4]. In contrast to genetic alterations, which are frequently identifiable via biopsy or sequencing, spatial heterogeneity necessitates sophisticated imaging and multi-omics methodologies to elucidate its intricacies. This comprehension has significant ramifications for precision medicine, wherein treatments are customised to the unique molecular characteristics of a patient's tumour [5]. Through the analysis of geographical distribution, physicians may effectively identify certain tumour locations exhibiting greater resistance to medicines, thus enhancing treatment efficacy and reducing the likelihood of relapse. Notwithstanding its increasing significance, the influence of spatial tumour heterogeneity on cancer resistance continues to provide a considerable challenge. Conventional diagnostic methods, mostly centred on bulk tumour specimens or single-site biopsies, frequently neglect to encompass the complete intricacy of spatial heterogeneity [6]. Consequently, these techniques may neglect essential subpopulations of resistant cells that could contribute to treatment failure. The absence of thorough methodologies for in vivo evaluation of tumour heterogeneity and the incapacity to dynamically monitor tumour response to therapy in real-time constitute significant constraints in contemporary cancer treatment frameworks. These problems highlight the necessity for research aimed at comprehending the spatial organisation of tumours and their contribution to resistance mechanisms [7].

This research seeks to investigate the nascent domain of spatial tumour heterogeneity and its role in cancer resistance. By examining the intricate relationship between geographical heterogeneity and therapeutic resistance, we aim to identify existing research gaps and suggest solutions to address these issues [8]. This review will illustrate how spatial heterogeneity, through an examination of recent advancements in imaging, omics technologies, and radiogenomics, might enhance the precision of therapy regimens for cancer patients. This work seeks to deliver a thorough examination of the potential of spatial tumour heterogeneity as a diagnostic instrument and therapeutic target in combating cancer. *Figure 1 provides a visual representation of spatial tumor heterogeneity, illustrating the diverse regions within a tumor that contribute to its resistance mechanisms and therapeutic challenges* [9].

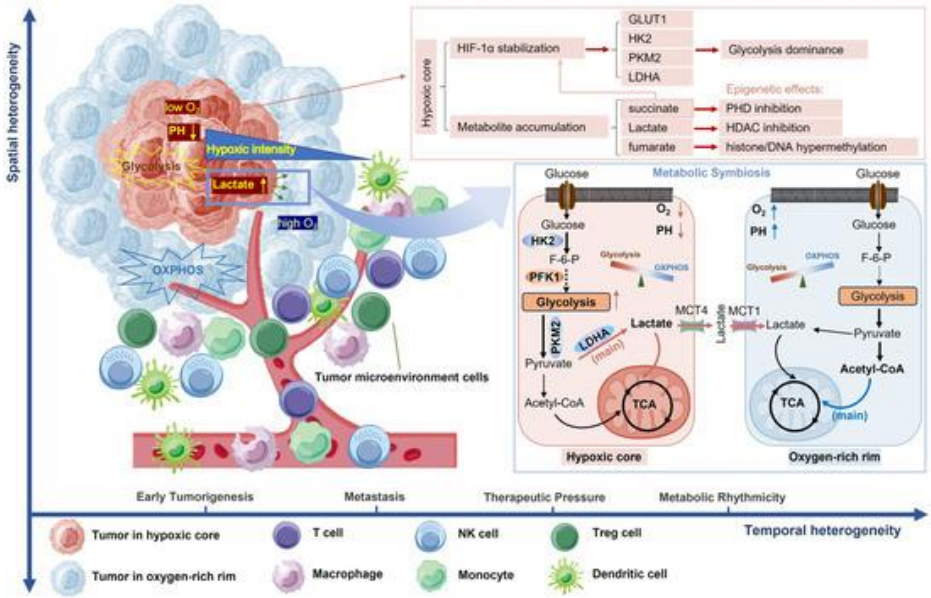

Fig 1: Spatiotemporal Heterogeneity of Tumor Glucose Metabolism Reprogramming: From Single-Cell Mechanisms to Precision Interventions. This fig delves into the dynamic reprogramming of glucose metabolism in tumors, emphasizing the importance of spatial and temporal heterogeneity. Advancements in single-cell sequencing, spatial omics, and metabolic imaging have shifted the paradigm from static analyses to high-resolution investigations of metabolic variations at the single-cell level. The article explores how these metabolic shifts influence tumor progression and response to therapies, highlighting the role of the glucose metabolism–epigenetics–immunology regulatory axis. By integrating these insights, the authors propose strategies for developing targeted interventions that address the complexities of tumor metabolism.

Summary of key points. Spatial tumor heterogeneity—differences in cell clones, perfusion, and immune contexture across regions—reliably predicts why some pockets resist therapy. We synthesize evidence across imaging, spatial-omics, and radiogenomics showing how these patterns map to hypoxia, immune exclusion, and drug delivery barriers. We outline practical workflows that convert those signals into decision-ready reports for tumor boards. Finally, we identify the main translational hurdles (standardization, validation, cost, and regulatory) and propose near-term solutions

The Role of Spatial Tumor Heterogeneity in Cancer Progression

Tumor Microenvironment (TME)

The tumour microenvironment (TME) is pivotal in the emergence of geographic heterogeneity in tumours. The tumour microenvironment (TME) comprises a complex network of cells, extracellular matrix constituents, blood arteries, and signalling chemicals that encircle and engage with tumour cells. This microenvironment is heterogeneous, differing both within various parts of the same tumour and among distinct tumours. Spatial heterogeneity emerges from the complex interactions among these tumour microenvironment components, which affect the tumor's biological behaviour and its treatment response [10].

A significant element influencing regional heterogeneity in the tumour microenvironment is hypoxia. Tumours frequently surpass their vascular supply, resulting in areas of hypoxia inside the tumour mass. Hypoxic areas, commonly situated near the tumor's centre, are generally marked by heightened resistance to chemotherapy and radiotherapy. Hypoxia activates multiple molecular pathways, notably the hypoxia-inducible factor (HIF) pathway, which facilitates tumour cell survival in oxygen-deficient regions by boosting angiogenesis, glycolysis, and various metabolic alterations. Besides hypoxia, immune cells in the tumour microenvironment contribute to geographic heterogeneity [11]. Immune cells, including T cells, macrophages, and dendritic cells, enter distinct tumour areas at varied densities. The presence and function of immune cells are frequently affected by the tumor's spatial design, which can either facilitate or impede tumor progression. The vasculature significantly influences the geographic heterogeneity of the tumour microenvironment (TME). The atypical blood arteries supplying tumours are generally permeable and chaotic, leading to inconsistent blood flow and the formation of areas with varying nutrition and oxygen concentrations. The disordered vasculature results in the development of

inadequately perfused regions, which contribute to the tumor's overall heterogeneity [12].

Clonal Evolution

Spatial heterogeneity is both a result of the tumour microenvironment and a catalyst for clonal evolution in cancer. Clonal evolution denotes the process through which tumour cells accumulate genetic changes over time, resulting in the formation of genetically different subpopulations, or clones, within an individual tumour. These clones have differing levels of aggressiveness, metastatic capability, and resistance to therapy, with their spatial distribution controlled by internal factors (e.g., genetic alterations) and extrinsic factors (e.g., the tumour microenvironment) [13].

Spatial heterogeneity promotes clonal evolution by creating diverse habitats inside the tumour, each characterised by unique selective pressures. Regions of the tumour subjected to elevated oxidative stress, hypoxia, or immune cell infiltration may promote the survival of clones more adeptly suited to these environments. This selection pressure propels the evolution of clones possessing genetic alterations that bestow resistance to treatments or facilitate metastasis. Research indicates that genetic heterogeneity within tumours correlates with poorer prognosis and more aggressive disease progression. In breast cancer, the existence of several genetically different clones inside a single tumour is associated with heightened metastatic potential and inferior patient outcomes. In glioblastoma, clonal evolution exacerbates the tumor's heterogeneity, complicating effective treatment. In these tumours, areas with elevated genetic alterations frequently exhibit greater resistance to standard therapies, whereas more genetically stable regions are less aggressive but may nonetheless play a role in tumour recurrence [14].

Intercellular Interactions

The interactions among various tumour cell types within the spatial environment additionally affect tumour growth and therapeutic resistance. These interactions are very dynamic, regulated by direct cell-to-cell contact and the production of soluble molecules that modify the behaviour of adjacent cells. In a tumour, peripheral cells may communicate with core cells via signalling molecules such as cytokines and growth factors, resulting in a heterogeneous tumour mass with diverse phenotypic traits. Intercellular interactions can enhance resistance mechanisms by fostering a more conducive milieu for specific cell populations [15].

The epithelial-mesenchymal transition (EMT) is a crucial signalling mechanism affected by geographical differences. Epithelial-mesenchymal transition (EMT) is a process whereby epithelial cells obtain mesenchymal traits, facilitating their migration and invasion into adjacent tissues. This mechanism is frequently linked to tumour advancement and metastasis. In tumours exhibiting spatial heterogeneity, epithelial-mesenchymal transition (EMT) is not equally active throughout all regions [16]. Cells in hypoxic or inadequately perfused regions may demonstrate a more pronounced epithelial-mesenchymal transition (EMT) phenotype, facilitating their invasion into adjacent tissues and the formation of secondary tumours. The PI3K/AKT pathway, which governs cell survival, proliferation, and metabolism, is significantly influenced by geographical heterogeneity. In select areas of the tumour, particularly those exhibiting elevated cellular density or distinct extracellular matrix constituents, PI3K/AKT signalling may be more intense, enhancing cell survival and facilitating resistance to apoptosis. The Wnt signalling pathway, integral to cell proliferation and differentiation, significantly contributes to tumour growth. In spatially heterogeneous tumours, the activation of the Wnt pathway may differ among various tumour locations, affecting tumour cell behaviour and contributing to resistance mechanisms [17].

The interplay between tumour cells and their microenvironment is additionally influenced by stromal cells, including cancer-associated fibroblasts (CAFs), which release extracellular matrix constituents and growth factors. These stromal cells establish a physical and metabolic barrier that affects drug distribution and the efficacy of treatments. In some areas of the tumour, cancer-associated fibroblasts (CAFs) may assist tumour cells in evading immune surveillance or augment the resistance of tumour cells to chemotherapeutic agents [18].

Spatial heterogeneity significantly influences tumour behaviour and treatment response. Spatial heterogeneity influences clonal evolution, intercellular connections, and signalling pathways, hence contributing to cancer growth, aggressiveness, and resistance, which is crucial for comprehending tumour biology and enhancing therapeutic options.

Mechanisms of Cancer Resistance Linked to Spatial Heterogeneity

Drug Resistance

Spatial heterogeneity significantly influences the emergence of treatment resistance in tumours. This resistance is not solely due to genetic changes but is frequently intensified by the spatial arrangement of tumour cells inside the tumour microenvironment (TME). Distinct tumour locations may have varied sensitivity to chemotherapy and targeted therapies,

mostly attributable to variations in oxygen availability, nutritional concentrations, cellular density, and the presence of stromal cells. In areas of the tumour characterised by significant hypoxia or inadequate nutritional availability, cells frequently experience heightened metabolic stress and demonstrate modified drug absorption, metabolism, and efflux, rendering them less vulnerable to standard therapies [19].

An illustrative instance of this phenomenon is evident in treatment resistance in solid tumours. In numerous malignancies, including glioblastoma and non-small cell lung cancer (NSCLC), the core hypoxic areas of the tumour demonstrate resistance to chemotherapy due to compromised drug delivery, modified cellular metabolism, and increased efflux pump activity. P-glycoprotein (P-gp), a membrane-associated transporter protein, is frequently increased in hypoxic areas, facilitating the efflux of chemotherapy agents such as doxorubicin, hence diminishing their efficacy [20].

Additionally, spatially disparate areas of the tumour may include genetically heterogeneous clones, each exhibiting different resistance profiles. In breast cancer, research indicates that the tumour periphery, characterised by superior oxygenation and vascularization, typically demonstrates a more favourable response to therapy, whereas the core displays increased resistance attributed to the hypoxic microenvironment and modified expression of drug-metabolizing enzymes. This varied medication sensitivity highlights the necessity for more advanced treatment techniques that consider the geographical heterogeneity within tumours. In targeted therapies, geographical heterogeneity can complicate treatment outcomes because of tumour subpopulations with unique genetic profiles. In HER2-positive breast cancer, tumour cells in hypoxic regions may downregulate HER2 expression, resulting in diminished responsiveness to HER2-targeted therapy such as trastuzumab [21]. These geographically different subpopulations can circumvent treatment by modifying critical signalling pathways or by acquiring mutations in therapeutic targets. *Figure 2 provides a conceptual overview of the various mechanisms contributing to drug resistance in different regions of the tumor, including hypoxia-driven resistance, immune evasion, and the emergence of resistant subclones* [22].

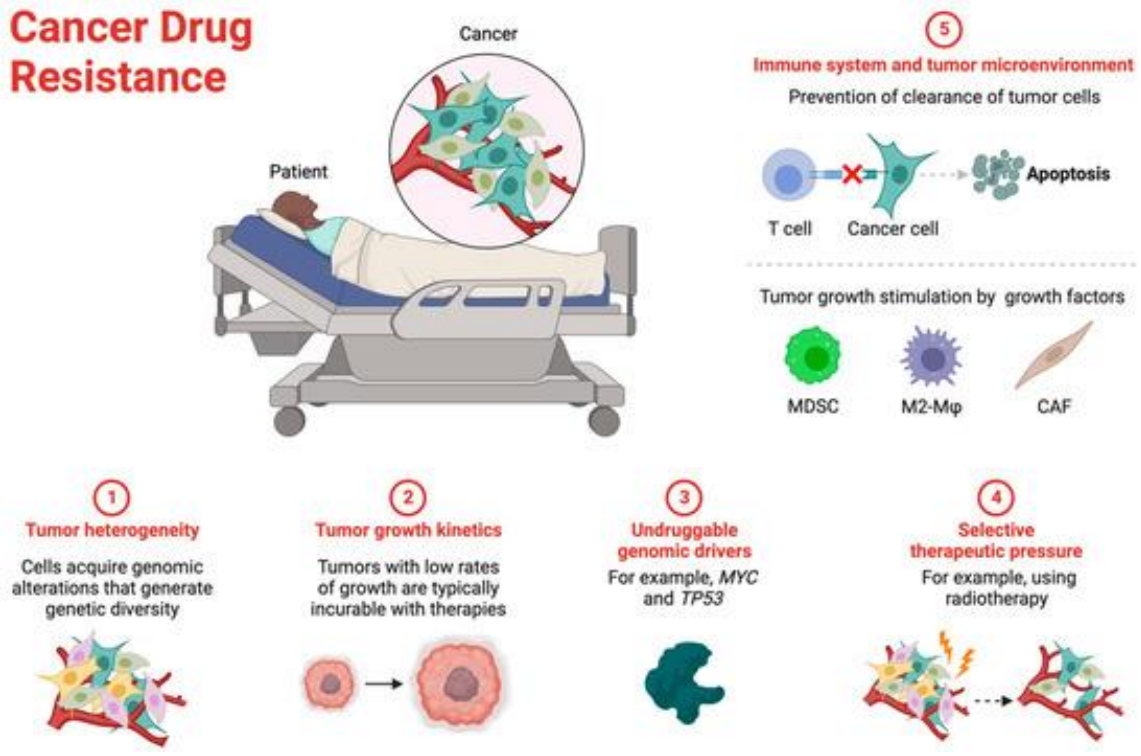

Fig 2: Multilevel Mechanisms of Cancer Drug Resistance. This figure explores the complex factors contributing to cancer drug resistance, including tumor heterogeneity, cancer stem cells, and the tumor microenvironment. It discusses how somatic mutations within tumors, enhanced DNA repair capabilities of stem cells, and interactions with immune cells and fibroblasts complicate treatment. Additionally, the article highlights how selective therapeutic pressures, such as radiotherapy, can induce resistance. The review emphasizes the need for combination therapies to overcome these multifaceted challenges in cancer treatment.

Immune Resistance

Spatial heterogeneity significantly influences immune resistance, since distinct tumour areas may display differing levels of immune cell infiltration and expression of immune checkpoint molecules. Immune surveillance, essential for identifying and eradicating tumour cells, may be hindered by the tumor's spatial layout, resulting in immune evasion in specific tumour locations. In numerous solid tumours, the immunological "cold" patches are characterised by restricted immune cell infiltration, typically located in the tumor's core. These regions are deficient in essential immune cells, including T cells, dendritic cells, and macrophages, required for a successful immunological response. Conversely, the immunological "hot" areas in the tumour perimeter frequently exhibit elevated levels of immune cell infiltration. Nonetheless, even in these immunodense regions, tumour cells can circumvent immune responses by upregulating immune checkpoint molecules such as PD-L1 (programmed death-ligand 1). These chemicals engage with their receptors on T cells (e.g., PD-1), resulting in T-cell fatigue and the inhibition of immunological responses. Immune checkpoint inhibitors are typically higher expressed in the peripheral layers of tumours, where immune cells are more prevalent, hence contributing to immune resistance in these areas [19,23].

An exemplary case of immune resistance associated with spatial heterogeneity is shown in melanoma, wherein immune checkpoint drugs such as anti-PD-1 treatment have demonstrated considerable therapeutic efficacy. Nonetheless, these medicines exhibit diminished efficacy in tumours characterised by diverse immune infiltration. Tumours characterised by areas lacking immune cells (cold tumours) demonstrate less responsiveness to immunotherapy, while places with increased immune infiltration (hot tumours) may show enhanced efficacy. Moreover, tumours exhibiting geographic heterogeneity might attract immunosuppressive cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), to specific areas, therefore further attenuating the immune response [24].

The capacity of tumours to circumvent immune surveillance in particular areas not only affects the efficacy of immunotherapy but also challenges the advancement of more effective immunotherapeutic strategies. Addressing geographically diverse immune evasion mechanisms may be essential to improve the overall efficacy of immunotherapy.

Therapeutic Implications

Comprehending spatial heterogeneity in tumours presents novel opportunities for enhancing cancer treatments, especially in addressing the obstacles of drug and immune resistance. A potential technique involves localised therapies that target the delivery of elevated medication concentrations or therapeutic agents directly to specific tumour areas. This can address the challenge of inadequate medication penetration in hypoxic or crowded tumour regions. Locally administered chemotherapies or nanoparticle-based drug delivery systems may offer tailored treatment for resistant tumour areas, enhancing therapeutic efficacy and reducing systemic adverse effects [25].

Another potential approach is combination therapy, which seeks to simultaneously address many facets of tumour resistance. Combining chemotherapy with immune checkpoint inhibitors may mitigate the resistance mechanisms in immunosuppressive tumour areas. Likewise, integrating targeted medicines that concentrate on certain biochemical pathways, such as PI3K inhibitors with HER2-targeted therapies, may effectively tackle the genetic and geographic heterogeneity of the tumour. Novel therapies also utilise tumor-stromal interactions to alter the tumour microenvironment. Stromal-modifying drugs that target cancer-associated fibroblasts (CAFs) or extracellular matrix components may affect the spatial configuration of tumour cells, hence enhancing their vulnerability to chemotherapy or immunological response. Moreover, techniques designed to reconfigure the tumour vasculature, including vascular normalisation therapy, may enhance the supply of chemotherapeutic drugs and immune cells to inadequately perfused tumour areas, hence improving treatment efficacy [26,27].

The emergence of spatial transcriptomics and radiogenomics enables the utilisation of spatial data from tumours to develop medicines specifically customised to the tumor's architecture. This data-centric methodology facilitates the creation of individualised treatment protocols that consider both the genetic and geographical characteristics of a patient's tumour, yielding a more accurate and efficacious therapeutic approach.

Comprehending the significance of spatial variation in cancer resistance offers a means to address substantial obstacles in cancer treatment. By focusing on spatially distinct tumour locations with localised therapies, integrating treatments, and employing breakthrough technology, we can more effectively tackle the intricate and evolving nature of tumour resistance, hence enhancing patient outcomes [28].

Techniques for Studying Spatial Tumor Heterogeneity

Imaging Techniques

Improvements in imaging tools have greatly enhanced our comprehension of spatial tumour heterogeneity, allowing researchers to observe the complex variations within tumour areas and their adjacent microenvironment. Conventional imaging techniques, including magnetic resonance imaging (MRI), positron emission tomography (PET), and computed tomography (CT) scans, have historically been utilised to examine tumour architecture and functionality. These imaging techniques offer macroscopic insights into tumour dimensions, morphology, and metabolic activity. MRI is extensively utilised to assess tissue architecture and the geographic distribution of tumour areas, especially beneficial for brain and soft tissue malignancies. PET and CT scans, although they provide insights into tumour metabolic activity and blood flow, exhibit insufficient resolution on detailed spatial heterogeneity at the cellular level [29].

Although conventional approaches yield significant insights, they are constrained in their ability to uncover cellular and molecular heterogeneities inside the tumour. In response, advanced imaging techniques have developed, facilitating enhanced resolution and more intricate visualisation of tumour heterogeneity. Multiplexed imaging facilitates the concurrent identification of many molecular targets inside tissue specimens, offering an extensive perspective of the tumor's spatial structure. This methodology integrates many imaging techniques, including immunofluorescence and mass spectrometry, to concurrently visualise various biomolecules inside a single tissue section, thereby providing insights into the heterogeneity of molecular markers, immune cell infiltration, and extracellular matrix constituents. Three-dimensional imaging is an advanced technique that produces three-dimensional reconstructions of tumour tissues, enabling researchers to examine the spatial arrangement of cells within the tumour with greater precision. Intravital microscopy is an advanced technology that facilitates the real-time observation of living tumour cells within intact tissues, offering a dynamic perspective on tumour progression, cellular migration, and therapeutic responses in their natural context. These sophisticated imaging techniques are essential for comprehending the influence of spatial heterogeneity on tumour proliferation and therapeutic resistance [30].

Omics Approaches

Besides imaging techniques, omics methodologies have emerged as crucial instruments for investigating spatial tumour heterogeneity. These methods amalgamate extensive molecular data, including genomic, transcriptomic, and proteomic information, to investigate the intricate interactions between tumour cells and their microenvironment. Genomic study reveals mutations, copy number variations, and chromosomal rearrangements that lead to tumour heterogeneity. When integrated with spatial data, genomic methodologies can identify certain tumour locations containing more aggressive or resistant clones [31].

Transcriptomics elucidates gene expression profiles across various tumour areas, emphasising the impact of cellular spatial distribution on their functional states. Single-cell RNA sequencing (scRNA-seq) is an innovative technique that facilitates the analysis of gene expression at the individual cell level. This approach can identify subpopulations of cells with unique transcriptional fingerprints, highlighting cellular diversity across various tumour areas. Single-cell RNA sequencing has demonstrated significant use in identifying unusual cell types that contribute to therapeutic resistance and metastasis [32]. Spatial transcriptomics is an emerging discipline that integrates gene expression profiling with spatial data, facilitating the mapping of gene activity inside tissue slices while maintaining the tissue's geographical context. This technique facilitates a high-resolution, localised examination of the molecular heterogeneity of tumours, elucidating the relationships between discrete tumour areas and their milieu, as well as the implications of these interactions for cancer growth and resistance [33].

Proteomics facilitates the examination of protein expression and alterations, yielding a functional assessment of the molecular processes occurring within various tumour areas. This method can assist in identifying resistance biomarkers and signalling pathways that are variably active in different tumour regions. Integrating proteomics with geographical data enables researchers to achieve a comprehensive understanding of the protein networks that regulate tumour heterogeneity and resistance mechanisms [34].

Computational Models

Computational models have become increasingly essential for analysing the extensive data produced by imaging and omics approaches in the investigation of spatial tumour heterogeneity. These models facilitate the amalgamation of many data kinds and offer robust instruments for forecasting tumour behaviour and therapy responses. One of the most sophisticated techniques is the creation of spatially resolved tumour atlases, which are comprehensive maps of tumours at the molecular, cellular, and tissue

levels. These atlases integrate spatial transcriptomics, imaging, and histology data to establish a complete reference for investigating tumour growth, resistance, and heterogeneity across many cancer types [35].

Machine learning algorithms are utilised to forecast tumour behaviour based on spatial heterogeneity. These models can amalgamate data from imaging, genetics, and proteomics to categorise tumour areas, anticipate therapy responses, and project clinical outcomes. Machine learning, by training algorithms on extensive datasets, can discern patterns that may not be immediately evident through conventional analysis, so facilitating more precise predictions of tumour responses to various therapies. This is especially beneficial in personalised medicine, where therapies can be customised to the unique spatial and molecular attributes of a patient's tumour [36].

Moreover, agent-based modelling and computational fluid dynamics are employed to mimic the dynamics of tumour proliferation, angiogenesis, and pharmacological administration in spatially heterogeneous tumours. These models integrate elements like as oxygen gradients, immune cell migration, and extracellular matrix reorganisation, offering insights into how these elements affect the tumor's spatial structure and its therapeutic response.

Collectively, these computational methods foster a more refined comprehension of spatial heterogeneity, hence aiding in the formulation of personalised treatment regimens aimed at specific tumour areas or biological pathways. By integrating computer models with experimental data, researchers can create dynamic, predictive models of tumour behaviour, thereby improving the creation of more effective and targeted medicines [37].

The integration of modern imaging techniques, omics methodologies, and computational models is facilitating substantial advancements in our comprehension of spatial tumour heterogeneity. These approaches offer critical instruments for identifying tumour subpopulations, elucidating resistance mechanisms, and formulating personalised treatment plans. As these technologies advance, they possess the capacity to transform cancer diagnosis, treatment, and prognosis, ultimately resulting in more efficacious medicines for patients [5].

Radiogenomics: Linking Spatial Heterogeneity with Imaging and Genomic Data

Definition of Radiogenomics

Radiogenomics is a nascent interdisciplinary domain that integrates radiomics (image-derived biomarkers) with genomic information to improve our comprehension of cancer at molecular and spatial dimensions. Radiogenomics seeks to combine sophisticated imaging methods with genetic data to discern tumour attributes that may be undetected by either approach independently. Radiogenomics has the potential to transform cancer diagnosis by providing a more thorough understanding of tumour heterogeneity, encompassing both genetic composition and geographic distribution of tumour cells. This data integration facilitates the mapping of genetic characteristics to distinct tumour locations, elucidating the impact of spatial heterogeneity on cancer growth and therapeutic response [38].

The principal benefit of radiogenomics in cancer detection is its capacity to offer a non-invasive method for finding and monitoring tumour heterogeneity. Conventional genomic analysis necessitates biopsy samples; however, imaging modalities like MRI, CT, PET, and multiplexed imaging can offer real-time insights into tumour architecture and metabolic activity without invasive procedures. Integrating genomic data with imaging modalities enables clinicians to examine the molecular foundations of spatial heterogeneity and perhaps forecast the treatment response of specific tumour locations, so providing a more accurate cancer management strategy [39]. *As shown in Figure 3, the integration of radiomic features from imaging data with genomic profiles provides a more comprehensive understanding of spatial tumor heterogeneity, allowing for better prediction of therapy response and resistance mechanisms* [40].

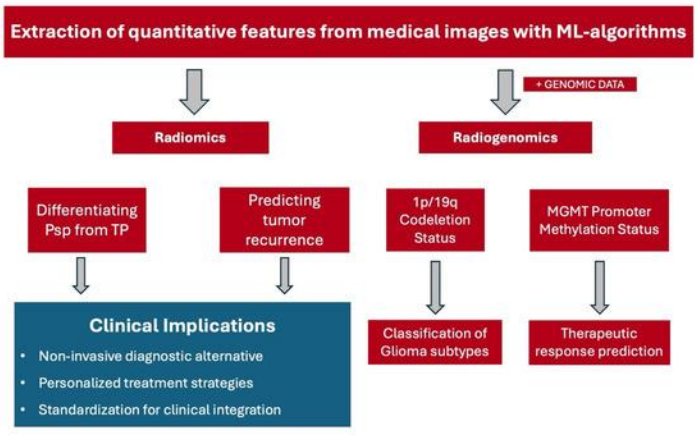

Fig 3: The Process of Radiomics and Radiogenomics in Comparison to Conventional Imaging Methods. This diagram visually represents the workflow involved in radiomics and radiogenomics methodologies, starting from image acquisition to diagnosis or clinical outcomes. It illustrates how the process integrates various data types, including genomic and omics data, after the initial image segmentation and data extraction. The comparison with

conventional imaging highlights the additional layer of genomic data integration in radiogenomics for a more comprehensive clinical assessment

Radiomics and Genomic Data Integration

The amalgamation of radiomics and genomic data facilitates the detection of regional patterns of tumour resistance that may remain undetected when utilising either modality in isolation. Radiomics entails the extraction of quantitative characteristics from medical imaging, including texture, shape, and intensity, which represent the underlying tumour microenvironment and its heterogeneity. These characteristics are essential in delineating tumour regions with varying biological behaviours, including locations exhibiting heightened vascularity, hypoxia, or cellular density. By linking these image-based traits with genomic data, researchers might obtain profound insights into the molecular foundations of tumour heterogeneity and resistance mechanisms [41].

Numerous studies have shown that radiomics can aid in identifying spatial patterns linked to medication resistance. In non-small cell lung cancer (NSCLC), radiomic characteristics such as tumour texture and morphology have been associated with distinct genetic subgroups, including those containing EGFR or KRAS mutations. These genetic modifications are frequently linked to specific resistance patterns to targeted medicines [42]. Zhang et al. (2024) showed that radiomic characteristics derived from CT scans of lung cancer patients correlate with molecular subtypes, offering significant predictive insights into therapy response and prognosis. Breast cancer research indicates that radiomic characteristics derived from MRI scans can forecast HER2 overexpression and other genetic markers, which correlate with certain therapeutic responses, such as to trastuzumab [43].

These investigations underscore the efficacy of integrating radiomic data with genetic information to pinpoint tumour locations that may contain resistant subpopulations. Besides genetic mutations, radiomics can elucidate alterations in the tumour microenvironment that foster resistance, including the formation of hypoxic regions or immune evasion niches. These locations may have unique radiomic signals that, when combined with genetic data, yield a more precise prediction of the therapeutic response of various tumour sites [38].

Clinical Applications

The clinical implementation of radiogenomics demonstrates significant potential in enhancing cancer treatment techniques, especially in predicting therapeutic responses and personalising treatment regimens. Radiogenomics is primarily utilised to forecast tumour responses to various treatments, such as chemotherapy, radiation therapy, and immunotherapy. By amalgamating radiomic characteristics with genomic data, doctors might discern tumours that are predisposed to respond to specific therapies based on their molecular profile and spatial heterogeneity [40].

Radiogenomics can predict the probability of tumour recurrence or metastasis by identifying tumour areas that exhibit greater treatment resistance. In glioblastoma, a highly heterogeneous and aggressive malignancy, radiogenomic methodologies have been employed to forecast the tumor's response to radiation therapy by correlating radiomic characteristics (such as tumour morphology and texture) with distinct molecular indicators, including MGMT promoter methylation status. Patients with tumours resistant to radiation can be identified early, facilitating more precise therapies, such as the administration of temozolomide or alternative chemotherapeutic drugs [44].

Radiogenomics is essential in formulating personalised treatment strategies. By integrating the geographical and molecular attributes of a patient's tumour, physicians can formulate more efficacious and personalised therapy. Radiogenomics can inform the selection of immunotherapies for tumours characterised by elevated immune checkpoint markers or distinct patterns of immune cell infiltration. By combining immune-related characteristics with genetic data, doctors can more accurately predict which patients are likely to benefit from immune checkpoint inhibitors, such as PD-1/PD-L1 inhibitors, hence avoiding ineffective treatments [44].

Moreover, radiogenomics possesses the capacity to enhance the assessment of therapy response. Therapeutic interventions allow for the monitoring of alterations in the tumor's molecular profile and geographic heterogeneity using successive imaging and genomic assessments. This facilitates real-time evaluation of therapy efficacy and may provide chances for early intervention upon detection of resistance. In ovarian cancer, radiogenomics has been employed to monitor alterations in the tumor's radiomic characteristics and molecular markers in response to chemotherapy, offering insights into the temporal evolution of various tumour locations.

In conclusion, radiogenomics presents an innovative methodology for cancer detection and treatment by amalgamating imaging and genomic data to elucidate the intricate geographic heterogeneity of tumours. This comprehensive approach offers significant insights into tumour resistance processes and has the potential to enhance therapy prediction, tailor treatment strategies, and assess therapeutic

response. As technologies progress, the capacity of radiogenomics to revolutionise cancer treatment will expand, providing patients with more accurate and efficacious therapeutic alternatives [45].

Future Directions and Challenges

Technological Advancements

The examination of spatial tumour heterogeneity is set for substantial advancement as new technologies progress. The urgent requirement is for the advancement of imaging techniques that provide enhanced resolution and greater penetration into tumour tissues. Contemporary imaging modalities such as MRI, CT, and PET scans offer significant insights; nonetheless, they frequently fall short in their capacity to delineate tumour microstructures at the cellular or molecular scale [46]. Advanced imaging technologies, like multiplexed imaging and 3D imaging, are enhancing spatial resolution; however, additional improvements are necessary to achieve more granular data. High-resolution intravital microscopy techniques offer potential for in vivo investigations by enabling real-time visualisation of tumour processes. Furthermore, the incorporation of these imaging modalities with data integration systems that amalgamate imaging, genomic, and proteomic data in real time will be crucial for comprehensively comprehending spatial heterogeneity and its influence on cancer progression and resistance.

Artificial intelligence (AI) is anticipated to be essential in the future of tumour spatial analysis. AI-driven algorithms can analyse extensive imaging and omics data to uncover concealed patterns and forecast tumour behaviour with greater precision. Utilising machine learning and deep learning methodologies, AI can improve picture spatial resolution, autonomously segment tumours, and associate spatial characteristics with molecular fingerprints, resulting in more accurate identification of tumour subpopulations and prediction of treatment efficacy. AI can aid in the creation of predictive models for tumour growth and therapeutic resistance, facilitating more targeted and individualised treatment strategies [47,48].

Challenges

Notwithstanding the promise of these technological breakthroughs, considerable technical obstacles persist in the acquisition and analysis of the intricate data related to spatial tumour heterogeneity. Tumours demonstrate significant complexity and variability, complicating the comprehensive capture of geographic heterogeneity through any singular technique. The amalgamation of varied data types, including imaging, genomics, and proteomics, constitutes a significant challenge, as each modality possesses distinct limits and necessitates advanced computational methods for integration [49].

The interpretation of data presents an additional challenge in the examination of spatial variability. Due to the substantial amount of data produced by imaging and omics approaches, standardisation challenges frequently occur in data analysis and interpretation. Inconsistencies in the methodologies employed for data collection, processing, and analysis can result in divergent outcomes, complicating the capacity to formulate universal conclusions or identify dependable biomarkers. Establishing standardised processes for data gathering and processing is essential for ensuring reproducibility and enabling multi-center investigations [50].

Clinical Translation

Despite considerable advancements in the laboratory, the application of these discoveries in clinical practice continues to pose difficulties. The clinical application of spatial tumour heterogeneity research necessitates the creation of more accessible and standardised diagnostic instruments suitable for widespread use in medical environments. Surmounting the financial and temporal obstacles linked to these new technologies is essential, as numerous existing approaches are resource-intensive and may not be practical for regular clinical application. Nonetheless, substantial potential exists for enhancing patient outcomes by addressing geographic variation in forthcoming medicines. By comprehending the responses of various tumour sites to treatment, clinicians can more effectively pinpoint spots necessitating intensified therapy or those with an elevated risk of recurrence. Customised treatment protocols that incorporate spatial heterogeneity may result in more efficacious cancer therapies with fewer adverse effects. Moreover, spatially-targeted therapies, including localised drug delivery and immunotherapy strategies aimed at resistant tumour areas, present significant opportunities for surmounting resistance and enhancing overall survival rates [51,52].

Despite the hurdles in technology and clinical translation, the future of investigating spatial tumour heterogeneity is highly promising. With the emergence of new technologies and an enhanced comprehension of tumour complexity, these improvements are expected to yield more accurate and effective cancer medicines, hence enhancing patient outcomes and transforming cancer care.

Future work should move beyond technology descriptions to **decision-changing evidence**. Priority

studies include prospective, habitat-guided biopsy trials (does imaging truly find discordant/resistant clones?), adaptive protocols that trigger early therapy switches based on week-3/6 imaging "habitat" response, and multi-site external validations with locked, IBSI-aligned pipelines to ensure calibration and reproducibility. Coupled, co-registered spatial omics can biologically validate what radiogenomic "habitats" mean, while pragmatic cost-effectiveness and equity audits (across scanners, sites, and demographics) will determine whether these approaches are affordable and fair at scale.

Conclusion

What can be integrated now (0–6 months).

1. **Standardize inputs:** adopt brief protocol cards for CT/MRI/PET (voxel size, kernel, normalization) and document segmentation with inter-reader agreement.
2. **Start with triage radiogenomics:** deploy a locked, IBSI-aligned feature set to flag hypoxia-like, immune-excluded, and high-cellularity "habitats" from routine scans—no new hardware required.
3. **Make it decision-ready:** generate a **one-page report** (habitats, suggested biopsy targets, therapy implications, trial flags) for tumor boards.
4. **Measure what matters:** track simple KPIs—time-to-treatment-switch, percentage of "non-diagnostic" biopsies avoided, and concordance with pathology.

Build reliability and scale (6–18 months).

5. **Prospective pilot(s):** pre-register a multi-site evaluation with fixed thresholds and calibration reporting; use decision-relevant endpoints (early progression under neoadjuvant therapy, time-to-switch).
6. **Selective spatial assays:** use imaging as a **triage** to decide *who* needs multiplex/spatial-omics and *where* to sample, reducing cost while increasing yield.
7. **Governance & transparency:** publish a brief model card (intended use, population, failure modes) and keep a version-locked pipeline for clinical evaluation.
8. **Training & handoff:** provide a 30-minute tumor-board walkthrough (how to read habitat maps; when to escalate or switch) and a checklist embedded in the report [53].

Health-system readiness.

These steps leverage existing PACS and pathology pipelines, require minimal additional staff time once templates are set, and focus investment where it changes decisions—biopsy targeting, early escalation, and rational combinations.

Limitations and next steps.

Harmonization across scanners, external validation, and reimbursement pathways remain essential. We recommend community adoption of IBSI-aligned features, shared reference datasets, and reporting standards that emphasize calibration and net clinical benefit.

Take-home.

By pairing standardized imaging with streamlined reporting and targeted confirmation assays, centers can begin using spatial heterogeneity within the next year to make earlier, better-informed decisions—bringing precision oncology closer to real-time practice [54].

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Review Article

Transforming Medical Laboratory Science with Vision-Language Models: A Focus on Microscopy in Microbiology, Hematology, and Histopathology

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Abstract:

Recent advances in artificial intelligence—particularly Vision–Language Models (VLMs)—offer promising avenues for enhancing microscopic diagnostics. This review synthesizes the current landscape of VLM applications across microbiology, hematology, cytology, and histopathology, spanning tasks such as Gram stain classification, cell-type recognition, feature localization, captioning, and report drafting. We outline how VLMs integrate visual features with domain-specific prompts to support triage, decision support, and quality control, while highlighting opportunities for few-shot and zero-shot generalization to rare findings. In parallel, we compare conventional convolutional pipelines with VLM-enhanced workflows, emphasizing gains in scalability, reproducibility, and explainability through multimodal rationales and grounded visual evidence. Key challenges include data curation and harmonization across laboratories, domain shift from variable staining and optics, bias and safety risks, limited task-relevant benchmarks, and the need for rigorous human-in-the-loop evaluation in clinical contexts. We propose a practical roadmap for deployment—covering dataset governance, prompt and template standardization, uncertainty reporting, and audit trails—alongside research priorities in robust evaluation, privacy-preserving learning, and alignment with clinical guidelines. Overall, VLMs are poised to complement expert microscopy by accelerating routine workflows and improving documentation, provided their adoption is guided by transparent validation and fit-for-purpose governance.

Keywords: Vision-Language Models (VLMs); Medical Laboratory Science; Microscopy; Artificial Intelligence; Diagnostic Automation

Introduction

Microscopy has historically been fundamental in medical laboratory diagnostics, providing direct visualization of cellular and microbial structures crucial for the identification of infectious, haematologic, and histopathological diseases. In microbiology, techniques such as Gram staining, Ziehl–Neelsen staining, and wet mounts offer swift initial assessments of bacterial, fungal, and parasitic infections. In hematology, the analysis of peripheral blood smears facilitates a comprehensive evaluation of erythrocyte and leukocyte morphology, assisting in the identification of anemia, hematologic malignancies, and platelet abnormalities. The histopathological assessment of stained tissue sections, especially with hematoxylin and eosin, aids in the analysis of architectural and cytological characteristics pertinent to malignancy, inflammation, and tissue degeneration. These routines are crucial to clinical decision-making, disease surveillance, and medical education [1].

Notwithstanding its diagnostic significance, conventional microscopy is limited by its manual characteristics and reliance on human proficiency. Variability in interpretation, operator tiredness, and training discrepancies contribute to diagnostic errors and diminished reproducibility. In high-throughput or resource-constrained environments, the scarcity of competent workers, protracted slide evaluations, and infrastructural inadequacies impede prompt and precise diagnosis. These issues show how important it is to have systems that can be scaled up, copied, and improved using computers to help people make better decisions and reduce workload bottlenecks.

Artificial intelligence (AI), especially deep learning approaches like convolutional neural networks (CNNs), has been useful in automating the process of finding patterns in medical images in domains like radiology, pathology, and cytology. However, CNNs usually give outputs that can't be understood, like classification scores or bounding boxes, because they can't put results in terms that are useful for therapy. Because of this limitation, there has been more interest in multimodal models that include an understanding of both text and images [2].

Vision-Language Models (VLMs) are a new type of AI that makes it possible to understand both images and words at the same time. Utilizing architectures like CLIP (Contrastive Language–Image Pretraining) and GPT-style language models, VLMs can produce structured descriptions or diagnostic narratives from visual input, so aligning more closely with the interpretive processes of laboratory specialists. A VLM can classify a Gram-stained image while simultaneously detailing cell morphology, staining characteristics, and probable organism classification—offering more comprehensive contextual information than unimodal models [3].

This article critically analyses the function of VLMs in enhancing diagnostic processes within three areas of medical microscopy: microbiology, hematology, and histopathology. We consolidate contemporary evidence from peer-reviewed research to evaluate the capabilities, performance measures (e.g., accuracy, AUC, F1 scores), and limits of current VLMs in these domains. Additionally, we tackle critical technological, ethical, and implementation difficulties, including data annotation quality, generalisability across varied clinical environments, privacy concerns, and regulatory monitoring. This review seeks to establish a practical and evidence-based framework for the incorporation of VLMs into clinical laboratory practice by contextualizing them within the larger scope of diagnostic automation. To elucidate the interpretative disparity between classic AI models and Vision-Language Models (VLMs), we present a visual example (Figure 1) that illustrates how each model type examines and conveys results from the identical haematologic smear. This contrast highlights the interpretability advantage of VLMs in generating elaborate, human-like narratives [4].

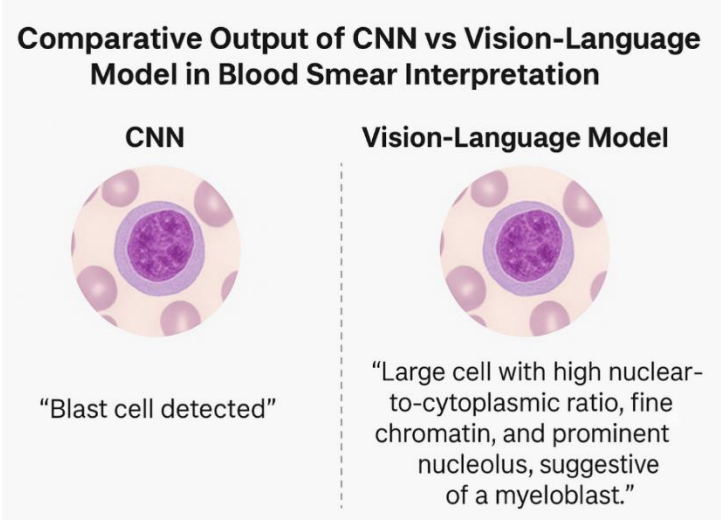
Comparative Output of CNN vs Vision-Language Model in Blood Smear Interpretation
CNN
Vision-Language Model
"Blast cell detected"
"Large cell with high nuclear-to-cytoplasmic ratio, fine chromatin, and prominent nucleolus, suggestive of a myeloblast."

Figure 1. Contrasting Outputs: Convolutional Neural Networks (CNNs) vs Vision-Language Models (VLMs) in Blood Smear Interpretation

This graphic displays a comparative analysis of outputs generated by a Convolutional Neural Network (CNN) and a Vision-Language Model (VLM) reading the same blood smear image. The CNN assigns a categorical label—"Blast cell detected"—whereas the VLM generates a comprehensible descriptive report: "Large cell with a high nuclear-to-cytoplasmic ratio,

fine chromatin, and prominent nucleolus, indicative of a myeloblast." This comparison underscores the narrative interpretability of VLMs, which emulate human diagnostic reasoning more proficiently than conventional CNN outputs.

Overview of Vision-Language Models (VLMS)

Vision-Language Models (VLMs) are a type of multimodal AI system that can interpret and combine visual and written input at the same time. Unlike traditional unimodal models that only work with images or text, VLMs combine these two types of data. This makes it easier to do things like captioning photos, answering visual questions, and reasoning across multiple modalities. These qualities make it easier to move from static categorization to outputs that are easier to understand. This lets models describe visual content in coherent natural language and connect it to contextual information.

Many different VLM designs work well on general-purpose datasets. Some of these models combine visual features with semantic text embeddings, while others add visual inputs to language models. In medical imaging, these features make it possible to find morphological patterns and make descriptive narratives that match the clinical record. For instance, when used on annotated pathology photos, VLMs can find features like cellular atypia or structural disorganization and present the results in structured prose that seems like a report from an expert [5].

This is different from standard convolutional neural networks (CNNs), which usually just give one output label without any context. Convolutional Neural Networks (CNNs) can correctly sort cell kinds or disease classifications, but they can't explain why they make the predictions they do. On the other hand, VLMs can give you a lot of information, including seeing Gram-positive cocci in chains and suggesting how to group them, or finding macrocytic erythrocytes next to hypersegmented neutrophils, which are signs of certain blood illnesses. These narrative outputs help with diagnosis and serve instructional and documentation purposes.

VLMs help with both pattern recognition and verbal expression of findings in microscopy-based diagnostics, which include microbiology, hematology, and histopathology. These are both important for laboratory reporting. Their outputs can help lab scientists by giving them a first look at things that need to be evaluated and validated, which could lower the number of different diagnoses and the time it takes to get results. Also, in places where resources are limited and there aren't enough trained specialists, VLMs may help with diagnosis by standardizing outputs and finding unusual patterns for experts to look at [6].

There are usually four steps to using VLMs in microscopy workflows: getting high-resolution digital images, processing them by the VLM to make structured descriptions, connecting with laboratory information systems for documentation, and having experts check the results before reporting. This method makes it easier to create a hybrid framework for AI-assisted diagnostics that puts a premium on transparency and reproducibility while having human oversight [5].

There are several possible benefits to using VLMs in microscopy, but their usage must be controlled by strict validation, clear performance standards, and appropriate regulatory frameworks. They are a useful tool for improving diagnostic procedures because they can produce standardized, understandable outputs. However, their real effectiveness depends on how well the model holds up, how good the training data is, and how well it works with current laboratory equipment. There are four steps to adding VLMs to microscopy workflows: getting high-resolution digital images, having the VLM automatically interpret them, making structured diagnostic descriptions, and having laboratory professionals check them. Figure 2 shows an example of this technique, which is a hybrid kind of human–AI collaboration in which automation helps but does not replace expert review. The goal of this system is to make it easier to get diagnostic services in both resource-rich and resource-limited settings, as well as to make them easier to repeat and shorten the time it takes to report [7].

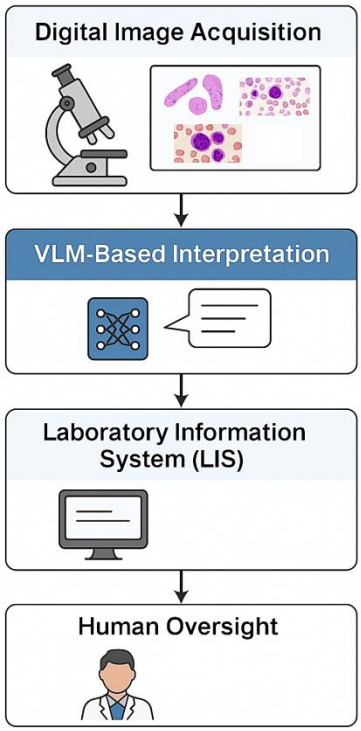

Figure 2. Workflow of Vision-Language Model Integration in Microscopy-Based Diagnostics. This figure illustrates the stepwise integration of a Vision-Language Model (VLM) into a digital microscopy workflow. It begins with image acquisition from stained slides (e.g., blood smears, Gram stains, histological sections), followed by VLM-based interpretation, where both image analysis and natural language reporting occur. The interpreted outputs are integrated into the Laboratory Information System (LIS), and final verification is conducted by a trained human expert. This hybrid human–AI workflow is designed to improve diagnostic efficiency, reproducibility, and scalability in clinical laboratory settings.

Utilization of Vision-Language Models in Microscopy

The application of Vision-Language Models (VLMs) in microscopy signifies a significant advancement in computational pathology, especially in the automation of intricate visual interpretation tasks that have historically relied on skilled human assessment. By integrating visual and textual modalities, VLMs create novel avenues for scalable and interpretable diagnostics. Nonetheless, their implementation necessitates a careful assessment of their practical accuracy, repeatability, and contextual constraints within laboratory settings. This section offers a specialized evaluation of VLM integration in microbiology, hematology, and histopathology.

Microbiology

Microscopy is essential for the prompt detection of infectious pathogens by techniques such as Gram stain and culture analysis. These procedures, although swift and informative, necessitate a sophisticated assessment of morphology, staining quality, and spatial arrangement—abilities that frequently differ based on experience and institutional training.

Clinical Workflow Example: Gram-stain triage (pre-/analytical/post-analytical).

Pre-analytical: After slide preparation and staining, the system ingests a low-magnification overview plus 2–3 high-power fields; specimen metadata (source, ward, recent antibiotics) is pulled from the LIS. **Analytical:** The VLM screens for "organisms present/absent," proposes Gram category and basic morphology (e.g., "Gram-positive cocci in clusters"), and produces a short rationale grounded in highlighted regions. **Targets:** Site-defined goals include ≥95% sensitivity for "organisms present," ≥90% specificity for negatives, and <60 s latency per slide to enable rapid triage. **Handoff: Positive** or **low-confidence** slides (e.g., high uncertainty or out-of-distribution alert) are queued to a scientist for confirmation; **high-confidence negatives** are batch-released for secondary review later in the shift. **Quality assurance:** Daily control slides are auto-scored; weekly audits trend sensitivity/specificity and review false-negatives. **Post-analytical:** Structured outputs (Gram call + rationale + ROI thumbnails) are appended to the LIS report with timestamps and versioned model IDs for traceability. **Impact:** The triage step reduces time-to-first-read for positives, cuts after-hours workload, and standardizes preliminary descriptions while keeping final sign-out with human experts.

VLMs trained on labeled Gram-stained images have shown the ability to produce structured descriptions that encapsulate bacterial morphology, organization, and staining properties. In controlled testing situations, many models attained classification accuracies of 90% in distinguishing common bacterial morphotypes. Nonetheless, difficulties remain in generalizing these models due to varied staining quality, unusual organisms, and imaging discrepancies, particularly in low-resource environments [1].

In addition to stain interpretation, colony morphology represents another domain where VLMs could improve reproducibility. Descriptors, including margin definition, pigmentation, and hemolysis patterns, frequently recorded inconsistently in manual

processes, can be systematically collected by models trained on annotated culture photos. However, significant intra-species heterogeneity and a scarcity of available datasets continue to pose technical challenges. Current research has not definitively established whether these algorithms can surpass expert microbiologists in atypical scenarios or in the presence of mixed illnesses.

VLMs provide the capability to automate zone diameter measurements and interpret breakpoints in accordance with guidelines for antimicrobial susceptibility testing (AST). Initial tests indicate over 85% agreement with human annotations in disc diffusion testing; however, performance deteriorates in cases of overlapping zones, indistinct edges, or uncommon infections [8]. Moreover, regulatory and process integration obstacles continue to pose substantial impediments to practical implementation. Figure 1 presents an organized diagram depicting the workflow from slide digitization to VLM-assisted interpretation and LIS integration, while Table 1 summarizes the comparative functionalities of VLMs against traditional methods across several areas.

Haematology

The study of peripheral blood smears is an essential diagnostic method in hematology for assessing erythrocyte morphology, leukocyte differentials, and platelet conditions. Manual review, while helpful, exhibits significant inter-observer variability and is susceptible to reader fatigue. VLMs trained on annotated smears have shown significant improvements in accuracy and interpretability [9].

A study examining six RBC morphologies showed that a vision-language architecture model attained a classification accuracy of 93.4% and an F1-score of 0.91 for sickle cell detection, metrics comparable to those of experienced hematologists. Significantly, VLMs extend beyond mere labeling by generating descriptive narratives, such as identifying hypersegmented neutrophils and associating them with macrocytic anemia patterns. These outputs improve traceability and diminish ambiguity in diagnostic communication [10].

Leukemic pathology adds additional intricacy. Blast detection, Auer rod identification, and left-shifted granulopoiesis recognition necessitate the integration of morphological context with diagnostic reasoning. Certain VLMs, when presented with expert-annotated pictures, have demonstrated the capacity to produce indicative interpretations; nonetheless, they necessitate validation via immunophenotyping and professional corroboration. In preliminary evaluations, metrics such as sensitivity and specificity have varied from 85% to 95%, contingent upon the model, cell type, and staining circumstances [11].

Notwithstanding these advancements, difficulties endure. Numerous datasets are constrained in terms of size, class equilibrium, and demographic heterogeneity. Furthermore, the majority of models have not been subjected to external validation in various laboratory environments, constraining their generalisability. The potential for overfitting to particular staining techniques or imaging resolutions also poses issues regarding transferability.

Histopathological Analysis

Histopathology is the most interpretatively challenging area of diagnostic microscopy. The process entails evaluating tissue architecture, cellular morphology, and context-dependent patterns, typically necessitating years of training. VLMs, when utilized on digitized whole-slide photographs, have demonstrated the capability to produce layered descriptions akin to initial diagnostic impressions [12].

For example, instead of categorizing an image merely as "adenocarcinoma," a VLM would articulate "proliferation of atypical glandular structures exhibiting mitotic figures and nuclear pleomorphism," accompanied by a diagnostic recommendation. In assessments of glandular histology, VLMs exhibit accuracy rates of 87–92% when compared to pathologist annotations, with inter-model variability frequently affected by the diversity of the training corpus and the grade of resolution [13].

In the assessment and classification of tasks—such as the allocation of Gleason or Nottingham scores—VLMs have generated structured outputs consistent with reference standards; nonetheless, error rates escalate in marginal situations and in tissue sections affected by inflammatory or necrotic variables. Restricted availability to high-quality, curated datasets for uncommon malignancies diminishes their robustness across the diagnostic spectrum.

Tasks related to biopsy interpretation, such as the identification of lymphovascular invasion, fibrosis, or necrosis, are inadequately investigated due to the complexities of data annotation. Moreover, interpretability in histology is essential; misdiagnosis may result in substantial clinical repercussions. Consequently, even slight advancements in automation necessitate corresponding stringent validation processes, quality assurance systems, and human oversight checks [14].

In an observational study of laboratory trainees, more than 80% deemed VLM-generated reports more useful than static atlas images [15]. Nonetheless, user happiness does not equate to diagnostic validity. Wider implementation must

emphasize regulatory supervision, transparency, and bias reduction—especially when models are developed using institution-specific datasets that may not represent the broader diversity of patients.

Table 1. Comparison of Traditional vs Vision-Language Model Methods in Microscopy.

Domain	Traditional Methods	VLM-Based Applications
Microbiology	Manual Gram stain interpretation and colony morphology documentation	Automated recognition and descriptive synthesis of Gram stains, colony features, and AST interpretations
Hematology	Manual differential counts; subjective morphology assessment	Automated cell classification with natural language reporting of abnormalities and differential suggestions
Histopathology	Pathologist-dependent tumor grading and morphologic interpretation	Structured, explainable outputs aligned with histologic grading and narrative reporting
Workflow Speed	Variable; dependent on expert availability	Real-time output generation; potential for streamlined reporting
Reproducibility	Prone to inter-observer variability	Consistent, model-driven output across repeated evaluations
Accessibility	Limited in settings lacking skilled personnel	Scalable across low-resource environments; supports telepathology

In summary, VLMs show strong promise in augmenting microscopy diagnostics, particularly in enhancing interpretability, improving workflow efficiency, and supporting underserved settings. However, enthusiasm must be balanced with caution. Rigorous external validation, performance benchmarking, and integration into regulated diagnostic frameworks are essential to ensure safety, generalizability, and equitable deployment.

Practical Benefits and Contextual Significance of Vision-Language Models in Microscopy

Vision-Language Models (VLMs) provide a novel approach to enhancing interpretability, consistency, and operational efficiency in microscopy-based diagnostics. Their incorporation into laboratory operations is motivated not solely by innovation but by the quantifiable benefits they provide in standardization, explainability, and scalability. This section rigorously analyses these benefits and delineates their practical significance, while also recognizing existing limitations and areas that need further assessment [16].

Standardization and Consistency in Diagnosis

One big problem with classical microscopy is that it relies on people to interpret the results. Inter-observer variability is still a big problem in diagnostic settings like Gramme stain, leukocyte morphology, and histologic grading, even for seasoned professionals. Vision-language models (VLMs) trained on image-text pairs that have been annotated by experts can help reduce this unpredictability by giving outputs that are structured and consistent [17].

VLMs have been able to correctly classify more than 90% of some morphologies in some areas, with F1-scores ranging from 0.85 to 0.93, depending on how hard the task is and how good the dataset is. Still, their results depend on the exact job and context; they may not work as well in rare situations, with atypical presentations, or with specimens that aren't stained well enough [18]. Because of this, VLMs improve the consistency of diagnoses, but they don't completely rid the need for expert validation or replace established quality assurance systems.

Understanding and Making Clear

Most traditional machine learning models, especially convolutional neural networks (CNNs), only give categorical labels or probability ratings, which might make it hard to put things in a clinical perspective. VLMs make it easier to understand by using clinical language to describe things in a way that makes sense. For example, instead of calling a cell a "blast," a VLM might describe its nuclear-to-cytoplasmic ratio, chromatin arrangement, and nucleolar prominence. This would provide a clear explanation similar to a pathologist's report [19].

This output format makes it easier to understand and more open in clinical settings. It also makes it easier to check for mistakes and encourages communication between lab staff, trainees, and doctors. Recent studies show that VLMs are better at speaking clearly, but they may not be as good at making diagnoses when the situation is unclear compared to human specialists. It's best to think of them as decision support tools rather than replacements for human skills [20].

Workflow Efficacy and Response Duration

Manual microscopy is intrinsically time-consuming. The diagnostic procedure may be prolonged, particularly in high-throughput laboratories or those experiencing personnel shortages, due to the time required for slide scanning and findings documentation. VLMs provide operational benefits by automating the analysis of standard specimens. In experimental installations, automated Gramme stain reporting utilizing VLMs decreased reporting times by more than 50%, with more significant enhancements noted in low-resource settings [21].

Nonetheless, the incorporation of VLMs into laboratory information systems and diagnostic reporting frameworks is complex. Concerns with picture preprocessing, system compatibility, and output standardization must be resolved before extensive implementation. The efficacy of VLMs is consequently linked to infrastructure preparedness and regulatory conformity.

Equity and Remote Diagnostic Assistance

A prominent advantage of VLMs is their capacity to facilitate diagnosis in environments with restricted access to expert microscopy. When integrated with digital slide scanners and cloud-based platforms, VLMs can deliver automated analyses in areas deficient in on-site professionals. Equity in diagnostic access relies not alone on algorithmic efficacy but also on internet connectivity, hardware availability, and language localization [22].

Presently, the majority of VLMs have been trained on datasets sourced from high-resource institutions, perhaps failing to encompass the complete range of clinical presentations observed worldwide. Rectifying this disparity necessitates intentional initiatives to mix training data and assess performance across geographically and demographically varied populations.

Standardization of Educational Support and Training

VLMs demonstrate potential as instruments for medical education. Their capacity to emulate expert analysis and elucidate morphological characteristics in natural language renders them advantageous in educational settings. In controlled investigations, trainees exposed to VLM-generated outputs reported enhancements in morphological comprehension and increased confidence in interpretative tasks [23].

However, the educational benefit must be assessed beyond subjective perceptions. Comparative studies assessing objective learning outcomes—such as pre/post-test performance or retention—are necessary to validate their educational efficacy. Furthermore, model explanations must conform to revised diagnostic criteria and refrain from perpetuating obsolete or inaccurate terminology, as noted in several initial implementations.

Although VLMs offer significant improvements to microscopy operations, their advantages must be understood in relation to their constraints. Diagnostic accuracy is extremely particular to tasks, generalisability is a challenge, and regulatory avenues for clinical application are still developing. Future investigations should emphasize prospective trials, external validations, and implementation in practical clinical environments. Only with such proof can the function of VLMs be distinctly defined and judiciously expanded within laboratory medicine [24].

Challenges in Implementation and Ethical Considerations

Notwithstanding the increasing interest in Vision-Language Models (VLMs), their application in clinical diagnostics is still in its infancy, with numerous unresolved technical, infrastructural, and ethical obstacles. Adoption has been inconsistent and largely restricted to experimental or pilot environments. A 2024 global audit of 520 healthcare facilities revealed that merely 14.7% have included generative AI tools into their diagnostic procedures, with less than 5% utilizing multimodal models like VLMs [25]. Most implementations were primarily situated in academic medical centers or AI-centric consortia, frequently bolstered by specialized bioinformatics teams and tailored infrastructure. The translation into wider clinical practice has been obstructed by apprehensions about model robustness, interpretability, medico-legal risks, and the sufficiency of existing regulatory frameworks [26].

Data Quality and Annotation Constraints

High-performance VLMs rely on extensive, meticulously annotated image-text pairs; yet, medical microscopy datasets are constrained, isolated, and inconsistently organized. In contrast to typical computer vision datasets (e.g., ImageNet), microscope images exhibit significant variability in staining techniques, magnification, resolution, and diagnostic classification. The creation of high-quality datasets, such as annotated Gramme stain panels, comprehensive peripheral smear narratives, or histopathology slide captions, generally necessitates laborious annotation by board-certified experts. Furthermore, semantic inconsistency—exemplified by terminological heterogeneity within institutions or inter-observer discrepancies in morphological classifications—exacerbates the challenges of dataset

standardization. These problems impede the scalability and generalisability of trained models [27].

Model Generalisability and Clinical Validation

VLMs trained on data from a restricted range of institutions or geographic areas frequently demonstrate inadequate external validity. The shape of erythrocytes may vary among communities due to endemic hemoglobinopathies or nutritional deficits. Histological characteristics may differ based on fixation methods or the anatomical site of the sample. In the absence of stringent cross-institutional benchmarking, models may underperform when used in unfamiliar clinical settings. Although numerous initial validation endeavors are present, such as the multi-center evaluation of the BioGPT-VL dataset on hematology smears (AUROC 0.84 ± 0.03, n = 4 locations), comprehensive, regulatory-grade investigations are still limited [28]. Consequently, model performance must be rigorously evaluated using varied, representative validation sets and made publicly accessible to guarantee clinical relevance.

Interpretability and User Confidence

Despite VLMs generating natural language outputs, their internal reasoning processes are not transparent. The risk of "hallucinations"—believable yet erroneous interpretations—has been recorded in recent assessments. A comparison study conducted by Yang et al. in 2025 revealed that GPT-4V exhibited a misunderstanding rate of 17.5% for unusual haematologic results, in contrast to 7.2% for pathologist consensus [29]. This disparity can undermine clinician confidence, particularly in critical situations. The use of interpretable characteristics, such as attention heatmaps, structured report templates, and uncertainty scores, may enhance transparency and promote safer implementation. End-user feedback mechanisms are crucial for rectifying model drift and enhancing confidence.

Data Privacy, Security, and Regulatory Compliance

Implementing and utilizing VLMs in healthcare environments presents considerable privacy risks. Microscopy pictures, although less innately recognizable than radiological scans, may nonetheless possess embedded metadata or be linked to uncommon diagnoses. Jurisdictions like the EU and the U.S. impose rigorous safeguards under GDPR and HIPAA, respectively. Moreover, cross-border model training, such as through federated learning or cloud-based fine-tuning, presents legal and ethical challenges concerning data sovereignty. Although technology precautions, including de-identification and encrypted pipelines, are progressing, they require enhancement through strong institutional control and compliance auditing. Regulatory bodies, such as the U.S. FDA and the European Medicines Agency, have not yet granted formal approval for any VLM as an independent diagnostic instrument; existing implementations are classified as "assistive" or "investigational," necessitating human monitoring [16].

Ethical Supervision and Human-Centric Integration

The ethical implementation of VLMs must reconcile the advantages of automation with protections against excessive dependence and skill degradation. Uncritically accepting automated microscopy results poses a risk of disseminating diagnostic errors, particularly in instances of rare diseases, unknown pathogens, or ambiguous morphologies. Clinical integration must emphasize hybrid workflows, wherein VLM outputs aid rather than supplant expert interpretation. Enhancing the capabilities of laboratory personnel, particularly in resource-limited environments, is crucial to guarantee that automation supports human decision-making rather than supplanting it. Moreover, institutional norms must explicitly define accountability in instances of discrepancies between AI-generated results and clinical outcomes [30].

Synopsis and Prognosis

The integration of VLMs into clinical microscopy is promising yet intricate. It is essential to address critical issues—dataset quality, generalisability, explainability, data governance, and ethical oversight—to go from research prototypes to regulated, real-world implementations. Effective execution necessitates interdisciplinary cooperation among clinical pathology, machine learning, health policy, and bioethics. Future endeavors must emphasize worldwide benchmarking initiatives, improvements in model transparency, and user-centric deployment frameworks to guarantee that VLMs promote diagnostic fairness instead of reinforcing current gaps [31].

Future Directions and Opportunities

Adding Vision-Language Models (VLMs) to medical laboratory operations opens up a number of strategic ways to improve both the accuracy of diagnoses and the training of the staff. One of the most useful short-term uses is in education and training. VLMs can be used as dynamic learning tools that let you add notes to microscope pictures in real time, make differential diagnoses, and create interactive Q&A

settings that mimic how experts think. In places where resources are limited and access to senior diagnosticians is limited, these models may be useful as scalable training tools. However, their effectiveness in teaching needs to be proven through rigorous evaluations of user performance before and after exposure [21].

Progress depends on having big, diversified, and expert-annotated microscopy datasets that are not just for education. Medical microscopy data is different from typical image-text datasets used in basic VLM training since the staining, resolution, pathology prevalence, and reporting methods vary from one institution to another. To make sure that everyone is included, people from all around the world need to work together on this. Federated learning and privacy-preserving data harmonization are two examples of projects that can help scale up data collection without putting patient privacy at risk [32].

Another important area for potential growth is systems integration. For VLMs to be useful in real-world diagnostic procedures, they need to work with both Laboratory Information Systems (LIS) and Electronic Health Records (EHR). Integration could make it possible to automatically highlight abnormal morphology, provide draft diagnostic narratives, and link patient histories or test findings across different types of data. Early pilot experiments have shown that it is possible to include massive language models in radiology report workflows. Similar frameworks may also work for pathology and microbiology [33].

Finally, future research should focus on explainability, regulatory preparedness, and human-AI collaboration. VLMs need to be made so that they can clearly explain why a diagnosis was made. For example, they may highlight parts of an image that affect the output or measure how sure they are. Adding feedback loops that let people fix or flag outputs will make things safer and more trustworthy. The ultimate goal is not to replace lab workers but to make decision-support systems that make human knowledge even better, especially in places where there are a lot of samples or not enough staff.

Conclusion

Vision-Language Models (VLMs) are a potential way to use computers for microscopy-based diagnostics because they combine extracting visual features with understanding plain language. Their use in microbiology, hematology, and histopathology may make it easier to automate descriptive reporting, sort through anomalous findings, and make sure that interpretative output is always the same. However, the present implementations are still in the testing phase, and clinical use is limited to pilot-scale deployments in institutions with a lot of resources.

There are many technological and institutional problems that need to be solved before it can be successfully integrated into clinical workflows. These include creating standardized, high-quality training datasets, thorough validation across several sites, strong systems for finding and explaining errors, and following changing rules and regulations for data governance. Without these protections, patient safety could be at risk due to things like model overfitting, diagnostic bias, or too much dependence on automation.

Also, the ethical use of VLMs must always have human monitoring, especially in unusual instances with new presentations or rare diseases. Collaboration between clinical labs, AI developers, and regulatory bodies from other fields will be necessary to make sure that VLMs improve, not replace, clinical reasoning. In the end, we should not see them as independent diagnosticians, but as cognitive aids that are part of human-centered diagnostic ecosystems. To find out how useful and reliable VLMs are in everyday clinical practice, further real-world studies will need to be done to look at their performance, how easy they are to understand, and how well they fit into existing workflows.

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Review Article

Prevalence and Predictors of Cervical Cancer Screening Uptake in Sub-Saharan Africa: A Systematic Review and Meta-Analysis

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Abstract:
Cervical cancer remains a leading cause of cancer-related mortality in Sub-Saharan Africa, where it disproportionately affects women due to late-stage diagnoses. Despite the availability of preventive measures such as screening and HPV vaccination, uptake remains critically low. This systematic review and meta-analysis aimed to estimate the pooled prevalence and identify key predictors of cervical cancer screening uptake across Sub-Saharan Africa. A comprehensive search of databases including PubMed, Google Scholar, African Journal Online (AJOL) and ScienceDirect identified 27 studies involving a total of 357,586 women. Our results revealed that the overall prevalence of cervical cancer screening uptake was 21.2% (95% CI: 16.2%, 27.2%). Key predictors of uptake included education, healthcare access, awareness of cervical cancer, age, and integration with other health services like HIV care. This study underscores the urgent need for tailored interventions to address barriers such as lack of awareness, financial constraints, and cultural stigma. The findings provide crucial evidence to guide policy and public health strategies aimed at increasing screening rates and reducing the cervical cancer burden in the region.

Keywords: Cervical Cancer; Cervical Cancer Screening; Sub-Saharan Africa; Screening Uptake; Public Health; Women's Health; HPV Prevention; Healthcare Access

Introduction

Cervical cancer remains a formidable public health challenge in Sub-Saharan Africa, where it is the leading cause of cancer-related mortality among women. The region accounts for approximately 85% of the global cervical cancer burden, with 18 out of the 20 countries with the highest rates located here [1]. In 2022 alone, Africa accounted for 23% of global cervical cancer mortality, translating to over 150,000 deaths [2]. This high mortality is primarily due to late-stage diagnosis, as most cases are detected at advanced stages when treatment options are limited and less effective [3].

The primary etiological factor for cervical cancer is persistent infection with high-risk strains of the Human Papillomavirus (HPV), particularly types 16 and 18. In Sub-Saharan Africa, the prevalence of HPV infection is notably high, and the progression from HPV infection to cervical cancer is expedited by factors such as early sexual debut, multiple sexual partners, and co-infection with HIV [4]. Despite the availability of preventive measures, including HPV vaccination and screening programs, the uptake remains alarmingly low in the region.

Cervical cancer screening is a crucial component of secondary prevention, aiming to detect precancerous lesions early when they are most treatable. However, the implementation of effective screening programs in Sub-Saharan Africa faces significant challenges. These include limited healthcare infrastructure, inadequate training of healthcare providers, and a lack of awareness among the population about the importance of regular screening. Additionally, cultural beliefs and stigma surrounding cervical cancer contribute to the reluctance of women to seek screening services [5].

The prevalence of cervical cancer screening uptake in Sub-Saharan Africa is dishearteningly low with estimates from various studies showing a pooled prevalence of approximately 12% to 14%[6]. This low uptake is attributed to various factors, including socioeconomic disparities, lack of access to healthcare facilities, and insufficient health education. Women in rural areas, those with lower educational levels, and individuals from lower socioeconomic backgrounds are disproportionately affected, highlighting the need for targeted interventions to address these inequities [7].

Understanding the predictors of cervical cancer screening uptake is essential for developing effective strategies to increase participation rates. Studies have identified several factors that influence screening behaviors, including age, education level, marital status, knowledge about cervical cancer, and access to healthcare services [8]. Moreover, integrating cervical cancer screening with other health services, such as HIV care and family planning, has been shown to improve screening rates by leveraging existing healthcare contacts [8].

By synthesizing data from various studies across the region, this research aims to provide a comprehensive understanding of the factors affecting screening behaviors. The findings are expected to inform public health policies and interventions aimed at increasing cervical cancer screening rates, ultimately contributing to the reduction of cervical cancer incidence and mortality in Sub-Saharan Africa.

Objectives and Research question

The objective of this systematic review and meta-analysis is to assess the prevalence of cervical cancer screening uptake in Sub-Saharan Africa and to identify the key predictors influencing this uptake.

The primary research question guiding this study is: "What is the prevalence of cervical cancer screening uptake in Sub-Saharan Africa, and what are the key predictors influencing this uptake?"

The framework for the research question follows the PIO (Population, Intervention, Outcome) format [7]. Where;

The PIO framework was adopted [9]:

- P (Population): Women in Sub-Saharan Africa
- I (Intervention): Cervical cancer screening
- (Outcome): Prevalence and predictors of screening uptake

Methodology

Study Design

This study followed the guidelines for meta-analysis outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [10].

Search Strategy

The literature search was conducted in four databases: PubMed, African Journals OnLine (AJOL), Google Scholar and ScienceDirect. The compiled search for all the databases were as below: (Cervical cancer OR Cervical malignancy) AND (Screening OR Assessment OR Examination) AND (Cervical cancer screening OR Cervical malignancy screening) AND (Uptake OR Utilization OR Participation) AND (Sub-Saharan Africa) AND (Prevalence OR Incidence OR Occurrence) AND (Predictors OR Facilitators OR Barriers). The search results were refined with the application of Boolean operators “AND” and “OR” [11]. MeSH terms were not employed in the search as the use of MeSH

terms retrieved few literature. The search focused on research articles published from 1st January 2010 to the search date (July 24, 2025).

Eligibility Criteria

Studies were included if they were original research articles that are observational studies (cohort, cross-sectional, case-control) published in English between 2010 to search date and reported on the prevalence of cervical cancer screening uptake among women in Sub-Saharan Africa. Studies focusing on populations outside Sub-Saharan Africa, those not reporting on screening uptake, or lacking data on predictors were excluded.

Study Selection

After screening the titles and abstracts of the retrieved studies, the full-text articles were assessed for eligibility. Two independent reviewers performed the screening to ensure consistency and minimization of bias. Disagreements between reviewers were resolved through consensus. Studies that met the eligibility criteria were included for data extraction.

Data Extraction and Quality Assessment

A data collection form was developed to systematically extract relevant information from the selected studies. This was conducted independently by two researchers and discrepancies were resolved through discussion. Key data extracted from the studies included information relating to study characteristics, sample size, screening prevalence, and identified predictors. The Newcastle-Ottawa Scale (NOS) risk of bias tool for observational studies was employed to assess the methodological quality of included studies [12]. The quality assessment was conducted independently by two researchers and discrepancies were resolved through discussion.

Data Analysis and Statistical Methods

Data were analyzed using Comprehensive Meta-analysis (CMA) software version 4. A random-effects model was applied due to anticipated heterogeneity across studies. Heterogeneity was assessed using the I^2 statistic and Cochran's Q test. Sensitivity analyses were conducted to test the robustness of the results. Funnel plots were used to visually map publication bias.

Results

Search results and Study Selection

A total of 30451 records were initially retrieved from multiple databases, including PubMed, ScienceDirect, Google Scholar, and AJOL. Following the application of appropriate filters, including the removal of duplicates, 29163 records were excluded before screening. After screening titles and abstracts, 1289 reports were eligible for title and abstract screening, but 1,253 were excluded based on exclusion criteria, leaving 36 reports for full-text review. Of these, 9 reports were ultimately excluded: 5 were review articles and 4 did not focus on prevalence or predictors of cervical cancer screening uptake. As a result, 27 studies were included in the final analysis [13-39]. The literature selection process is illustrated in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram as shown in Figure 1.

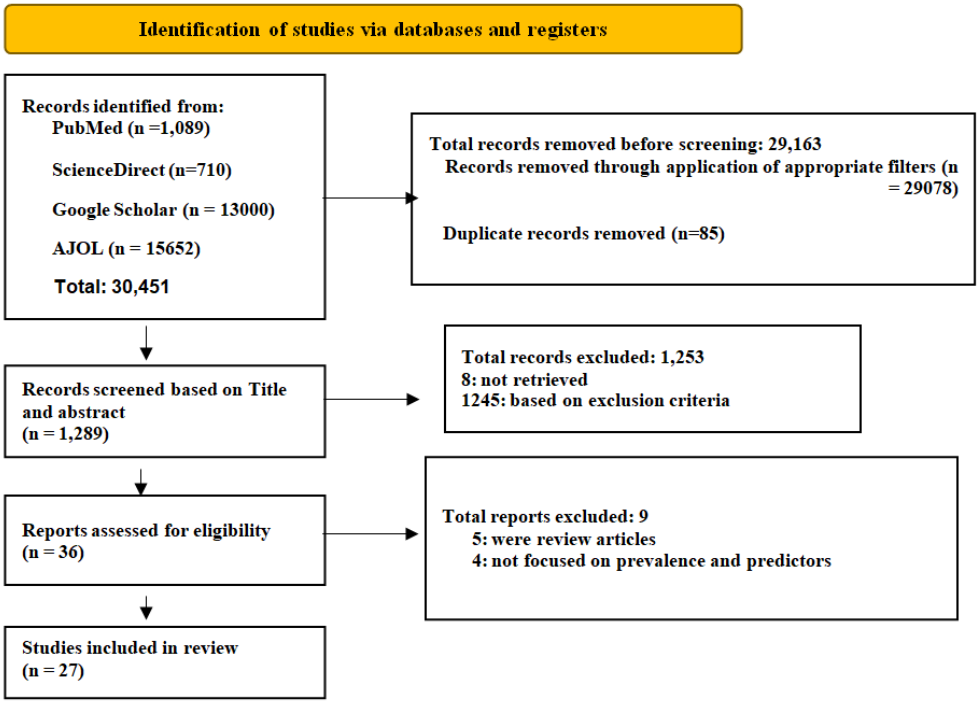
Identification of studies via databases and registers
Records identified from:
PubMed (n =1,089)
ScienceDirect (n=710)
Google Scholar (n = 13000)
AJOL (n = 15652)
Total: 30,451
Total records removed before screening: 29,163
Records removed through application of appropriate filters (n = 29078)
Duplicate records removed (n=85)
Records screened based on Title and abstract (n = 1,289)
Total records excluded: 1,253
8: not retrieved
1245: based on exclusion criteria
Reports assessed for eligibility (n = 36)
Total reports excluded: 9
5: were review articles
4: not focused on prevalence and predictors
Studies included in review (n = 27)

Figure 1: PRISMA Flow Diagram

Study Characteristics and Quality Assessment

The characteristics of the included studies in this review varied in their design, sample size, and participant characteristics as shown in Table 1, reflecting the diverse contexts of cervical cancer screening uptake across Sub-Saharan Africa. Study designs were predominantly cross-sectional, with a few retrospective cohorts and mixed-methods approaches. There are a total of 357586 women studied in all the included studies and sample sizes ranged from as few as 163 participants to over 124,000, with the majority involving women aged 25-49 years. The studies focused on various populations, including general female populations, HIV-positive women, and women attending healthcare facilities. The prevalence of cervical cancer screening uptake varied widely, with figures ranging from 4.3% to 89.9%, highlighting substantial disparities in screening coverage across the region. Key facilitators identified included education, income, healthcare access, and awareness campaigns, while barriers often included lack of awareness, financial constraints, and cultural beliefs.

Quality assessment using the Newcastle-Ottawa Scale as shown in Supplement File 1 revealed variability in study quality. Most studies were rated highly for selection criteria but were often limited by insufficient comparability or incomplete outcome assessments. Several studies had moderate to high ratings in terms of exposure ascertainment, but follow-up duration and completeness varied, with some studies lacking comprehensive follow-up, which may impact the robustness of their findings.

Table 1: The characteristics of the included studies

Study ID	Country	Study Design	Total number of participants	Age of Participants	Population Studied	Prevalence	Predictors (Facilitators — Barriers)	Source Journal
Ngwu et al. (2022) [13]	Nigeria	Cross-sectional Mixed-method study(quantitative & qualitative)	517 women	Majority aged between 25 and 34 years	Women in Enugu State, Nigeria	89.9% (465 out of 517)	Barriers: Education, income, residence; Facilitators: Social worker involvement	Asian Pacific Journal of Cancer Prevention
Pry et al. (2021) [14]	Zambia	Cohort	183 165women	median age of women was 34 years	Women in Zambia (Health Facilities)	36.65% (67132 out of 183,165)	Facilitators: Age, HIV status, income	The Lancet Global Health
Arage et al. (2025) [15]	Multiple SSA Countries	Retrospective Cross-Sectional study	75,360 women	Majority aged between 25–49	Women across 10 SSA countries	51.63% (38,907 out of 75,360)	Facilitators: Wealth, contraceptive use, media exposure; Barriers: Lack of access to healthcare	BMC Medical Informatics and Decision Making

Hailegebireal et al. (2024) [16]	Eleven (11) SSA Countries	Retrospective Cross-Sectional study	124,787 women	mean (± SD) age of 29.38 (± 8.61) years	Women across 11 SSA countries	10.29% (12,849 out of 124,787)	Facilitators: Age, education, wealth, health access; Barriers: Lack of awareness, financial constraints	BMC Cancer
Gemeda et al. (2020) [17]	Ethiopia	Cross-sectional	838 women	Participants were 25 years and above	Women aged 25 years and above in Sidama Zone, Southern Ethiopia	17.8% (149 out of 838)	Facilitators: Knowledge, HIV status, perceived self-efficacy; Barriers: Lack of awareness, financial constraints	Cancer control : journal of the Moffitt Cancer Center
Yasin et al. (2024) [18]	Ethiopia	Cross-sectional	396 HIV-positive women	Participants were 20 to 49 years, mean age was 32.8 (± 8.68) years	HIV-positive women in Asella Town, Ethiopia	30.3% (120 out of 396)	Facilitators: Knowledge, attitude, health worker sensitization; Barriers: Fear of positive test results	BMC Infectious Diseases
Baykemagn et al. (2025) [19]	Ghana, Kenya, Mozambique, and Tanzania	Retrospective Cross-Sectional	33,952 women	Not reported	Women aged 30-49 in SSA countries	13% (4,414 out of 33,952)	Facilitators: Wealth status, HIV test history, education; Barriers: Lack of access to healthcare	JMIR Public Health and Surveillance
Okyere, Ayebeng, & Dickson (2024) [20]	Côte d'Ivoire	Cross-sectional	9,078 women	Participants were 25 to 49	Women aged 25-49 in Côte d'Ivoire	7.52% (683 out of 9,078)	Facilitators: Age, education, wealth, health insurance; Barriers: Financial constraints, lack of awareness	BMC Health Services Research
Isabirye, Mbonye, & Kwagal	Uganda	Cross-sectional	845 women	Participants were 25 to 49, majorit	Women aged 25-49 in Central Uganda	20.6% (174 out of 845)	Facilitators: Knowledge, wealth, health worker sensitization; Barriers: Lack of awareness, financial constraints	PLOS ONE

a (2020) [21]				y were aged 25–39 years				
Mungo et al. (2024) [22]	Kenya	Retrospective Cross-Sectional	57,298 HIV-positive women	Mean age = 31.4 years	Women living with HIV in care programs, Western Kenya	29.4% (16,846 out of 57,298)	Facilitators: Age at first screening, ART use; Barriers: Lack of funding, healthcare infrastructure	JCO Global Oncology
Wambui et al. (2024) [23]	Kenya	Cross-sectional study	391 women	Participants aged 15-54 years, Mean age = 34.5 years	General public, Women in Kiambu Sub-County in kenya	25.8% (101 out of 391) screened	Facilitators: Education level, Religion (Muslim) Barriers: Low income, Misconceptions	East African Medical Journal
Ehwarieme & Emina (2022) [24]	Nigeria	Cross-sectional study	220women	Mean age = 30 years	Female teachers in Nigeria	10.45% (23 out of 220) screened	Facilitators: Educational level, knowledge; Barriers: Lack of access, cost concerns	The Nigerian Health Journal
Okyere (2025) [25]	Kenya	Cross-sectional	16,824 women	15-49 years	Women of reproductive age from Kenya Demographic and Health Survey	16.68% (2,837 out of 16,824) screened	Facilitators: Multiple sexual partnership (MSP); Barriers: Low education, urban vs rural differences, distance to healthcare, wealth disparities.	BMC Cancer
Bogale, Ali, & Sherif (2024) [26]	Ethiopia	Cross-sectional	578women	≥25 years	HIV-positive women in Addis Ababa, Ethiopia	31.5% (182 out of 578) screened	Facilitators: recommendation from health professionals; higher income; certain occupations; longer duration living with HIV. Barriers: consider themselves healthy; perceived screening as painful; feeling shy to be screened; inadequate knowledge	Cancer control : journal of the Moffitt Cancer Center,

Woldetsadik et al. (2020) [27]	Ethiopia	cross-sectional study	425 women	18–49 years	Women attending family health department (outpatient visitors)	12.2% (52 out of 425)	Facilitators: heard about screening benefits; knowledge of screening place; perceived risk. Barriers: rural residence; low monthly income; lack of knowledge; fear of test outcome; lack of information/ not knowing place of service; feeling healthy.	BMC Women's Health
Emru, Abebaw, & Abera (2021) [28]	Ethiopia	Cross-sectional	411 women	15-49 years	HIV-infected women	25.5%(105 out of 411) screened	Facilitators: Awareness of cervical cancer and screening. Barriers: Lack of awareness, fear of positive results, fear of painful procedures, long waiting times.	Women's Health
Adzigbli et al. (2025) [29]	Ghana	Cross-sectional	7,124 women	30-49 years	General female population	7.27% (517 out of 7,124)	Facilitators: Higher education, media exposure, health insurance, visits to health facilities. Barriers: Low wealth index, rural residence, lack of awareness.	BMC Infectious Diseases
Tsekpetse et al. (2025) [30]	Ghana	Cross-sectional	7,105 women	30-49 years	General female population	6.83% (485 out of 7105)	Facilitators: Higher education, health facility visits, media exposure, higher wealth index. Barriers: Rural residence, lack of health insurance.	BMC Women's Health
Fentie, Tadesse, & Gebretekle (2020) [31]	Ethiopia	Cross-sectional Mixed-method study(quantitative & qualitative)	844 women	Mean age: 35.74 (±7.6) years	Women attending cervical cancer screening services in Addis Ababa, Ethiopia	10.3% (87 out of 844)	Facilitators: Age, knowledge of cancer, access to services; Barriers: Lack of awareness, cultural/religious beliefs, stigma, inaccessibility.	BMC Women's Health
Njoroge et al. (2023)	Kenya	Mixed-method, cross-sectional	272 women	18-59 years	Women in Laikipia East Sub-County, Kenya	32.4% (88 out of 272)	Facilitators: Age, income level, awareness campaigns; Barriers: Fear, lack of time, stigma, lack of	African Journal of Health Sciences

[32]							knowledge on benefits of screening.	
Saasa-Modise et al. (2022) [33]	South Africa	Descriptive Cross-sectional study	550 women	23-68 (median: 34)	HIV-positive women on ART in primary health care clinics	32.9% (181 out of 550)	Facilitators: ART adherence; Barriers: Lack of staff training, delayed screening, poor policy adherence, low screening uptake in younger women.	Pan African Medical Journal
Isara & PC-Onyekwere (2024) [34]	Nigeria	Cross-sectional study	214 women	15-49 years	Antenatal clinic attendees	16.2% (31 out of 191)	Facilitators: Awareness (via health workers, mass media); Barriers: Fear of pain, lack of perceived susceptibility, lack of awareness of symptoms, financial constraints, and fear of positive results.	Journal of Medicine & Biomedical Research
Ngutiku, Mwenda, & Maranga (2021) [35]	Kenya	Descriptive Cross-sectional study	163 women	20-59 years	Nurses at Thika Level 5 Hospital	41% (67 out of 163)	Facilitators: Knowledge of cervical cancer; Barriers: Fear of test results, lack of perceived susceptibility, and the belief that cervical cancer screening is unnecessary for young nurses.	East African Medical Journal
Omowhara, Ameh, & Banjo (2022) [36]	Nigeria	Cross-sectional	234 women	30–65 years; mean 41.08 ± 8.45 years	Adult women resident in a rural community (Orhuwhorun) — community household	4.3% (10 out of 234)	Facilitators: Health education / increased awareness; availability of affordable & accessible screening. Barriers: Lack of awareness/knowledge, lack of spousal support, misperceptions/stigma/modesty, cultural beliefs/traditions, cost of screening, limited access	The Nigerian Health Journal.
Kangethe et al. (2022) [37]	Kenya	Cross-sectional Mixed-method study(q	305 women	Median age = 36 years	Women living with HIV (WLHIV) receiving HIV care	44.6% (136 out of 305)	Facilitators: Older age , higher education, knowledge of the HPV vaccine. Barriers: User fees/costs, fear of painful/invasive	Southern African Journal of HIV Medicine

		uantitative & qualitative)			at the KNH Comprehensive Care Center		procedure and perceived effect on fertility, provider characteristics, long waiting times, stock-outs	
Olatubi et al. (2024) [38]	Nigeria	Descriptive cross-sectional	313 women	Mean 22.39 years; majority 21–25 years	Female undergraduate students at a Nigerian university	9.3% (29 out of 313)	Facilitators: Willingness to be vaccinated; opportunistic screening by healthcare workers recommended by authors. Barriers: Lack of awareness, cost/expense, ignorance of need, not knowing where to get screened	Bayero Journal of Nursing & Health Care.
Ndateba, Kabatsinda, & Ndabarora (2021) [39]	Rwanda	Cross-sectional	178 women	Adults ≥25 years	Women aged ≥25 years attending outpatient department at Rwamagana Hospital	23.0% (41 out of 178)	Facilitators: Higher knowledge and higher monthly income Barriers: low knowledge, poverty, lower education, rural residence, limited access	Rwanda Journal of Medicine and Health Sciences V

Meta-analysis Findings

Forest Plot Analysis

The forest plot analysis of the included studies as shown in Figure 2 reveals considerable variability in the event rates (uptake of screening) of cervical cancer screening uptake across Sub-Saharan Africa. The event rates in the included studies ranged from 0.023 to 0.899, whereas the pooled event rate, as indicated by the random effects model, was 0.212 (95% CI: 0.162, 0.272). Therefore, the overall prevalence of cervical cancer screening uptake in Sub-Saharan Africa based on the included studies, as determined by the random effects model was 21.2% (95% CI: 16.2%, 27.2%). This pooled estimate of 21.2% indicates that cervical cancer screening uptake remains low across the region. Each study's point estimate is represented by a square, and the 95% confidence intervals are shown by the horizontal lines.

Meta-Analysis Statistics and Heterogeneity

According to the statistical indices, as shown in Figure 3, the fixed-effect model yields a point estimate of 0.313 (95% CI: 0.312, 0.314), which is slightly higher than the random-effect model's estimate of 0.212 (95% CI: 0.162, 0.272). This disparity indicates significant between-study heterogeneity. The high Tau-squared value of 0.862 and the I-squared value of 99.948% further highlight the substantial variability across the studies. Such heterogeneity may be attributed to differences in study design, population characteristics, and regional disparities in cervical cancer screening practices.

Distribution of True Effects

Figure 4 illustrates the distribution of the true effect size for cervical cancer screening uptake, with a pooled estimate of 0.212 (95% CI: 0.162, 0.272). This suggests that, for a typical population in Sub-Saharan Africa, the expected screening uptake falls within this range. The 95% prediction interval spans from 0.04 to 0.62, indicating that while most similar populations are expected to have screening uptake close to the mean, the true effect size for each study remains uncertain. This wide interval reflects the substantial variability across the studies included in the analysis.

Funnel Plot Interpretation

The funnel plot presented in Figure 5 provides a visual assessment of the standard error plotted against the logit event rate for studies included in this

meta-analysis. The plot suggests a symmetrical distribution of data points, indicating the absence of significant publication bias. The shape appears to follow an expected funnel pattern, with smaller studies (greater standard errors) scattered towards the bottom, while larger studies (smaller standard errors) concentrate near the top. This symmetry strengthens the reliability of the overall estimates and supports the robustness of the findings regarding cervical cancer screening uptake in Sub-Saharan Africa.

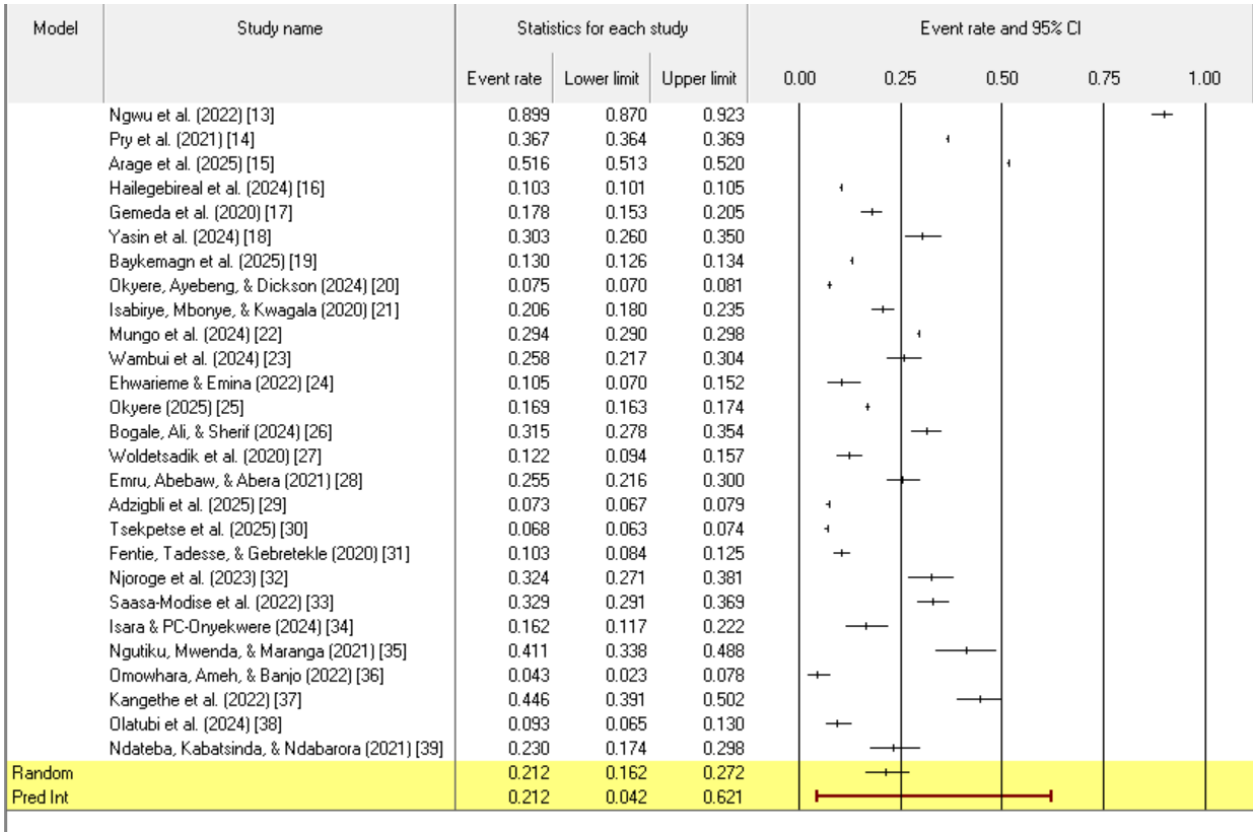

Figure 2: Forest Plot

Model	Effect size and 95% interval			Prediction Interval		Between-study		Other heterogeneity statistics			
Model	Number Studies	Point estimate	Lower limit	Upper limit	Lower limit	Upper limit	Tau	TauSq	Q-value	df (Q)	P-value	I-squared
Fixed	27	0.313	0.312	0.314					50210.699	26	0.000	99.948
Random	27	0.212	0.162	0.272	0.042	0.621	0.862	0.743				

Figure 3: Meta-analysis statistics

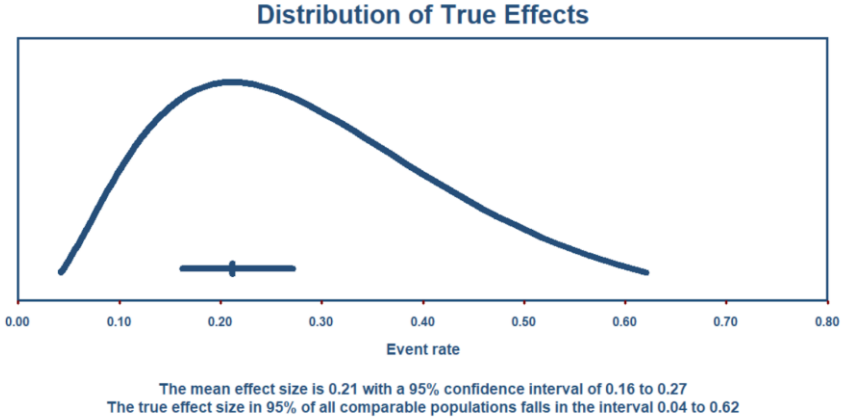

Figure 4: Distribution Plot of True effects

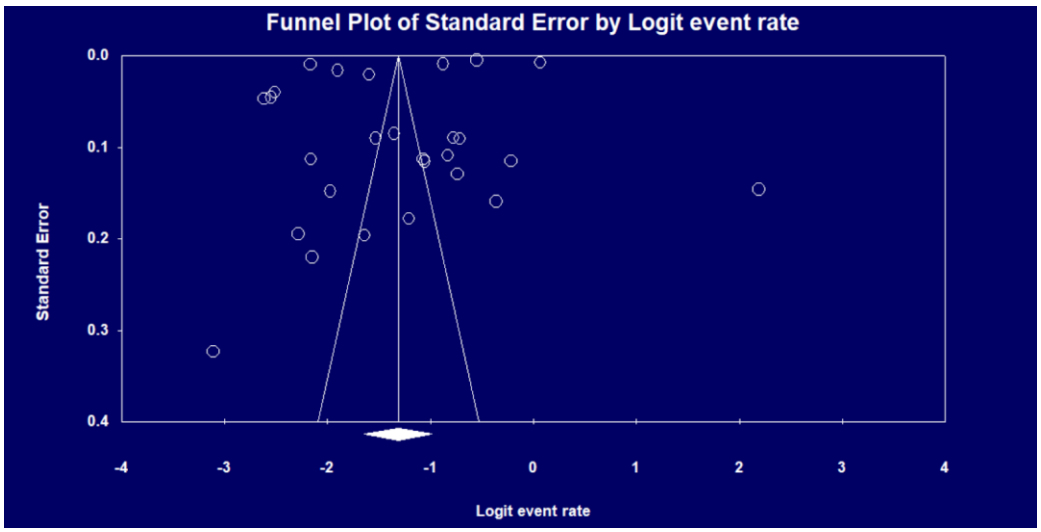

Figure 5: Funnel Plot

Narrative Synthesis for the Predictors of Cervical Cancer Screening Uptake

This section explores the predictors (facilitators and barriers) of cervical cancer screening uptake, based on findings from 27 studies included in this review. The information synthesized here reveals significant trends, variations, and disparities in the factors influencing screening uptake across different demographics, regions, and healthcare settings.

Facilitators of Cervical Cancer Screening Uptake

I. Knowledge and Awareness of Cervical Cancer

A critical facilitator of cervical cancer screening uptake is knowledge about the disease, including its risk factors, symptoms, and the benefits of early detection. Several studies underscore the importance of health education in improving screening rates, highlighting that women who are more aware of cervical cancer and its prevention methods are more likely to participate in screening programs. For instance, studies in Kenya found that increased knowledge about the risks of cervical cancer and the role of screening in early detection significantly correlates with higher screening uptake [21, 24]. Women who are informed about cervical cancer's preventability through screening are more likely to take part in programs aimed at early detection.

The studies also suggest that knowledge about the importance of early screening is more prevalent among women who have received health education either through formal healthcare settings or community outreach programs. In rural areas, however, knowledge gaps persist, leading to low participation rates in screening. For example, studies conducted in rural Ethiopia [26], and Nigeria [36] report a significant lack of awareness about the availability of screening services, contributing to lower uptake.

Furthermore, the effectiveness of education campaigns varies by demographic factors such as education level and location. For example, a study by Omowhara (2022) [36] in Nigeria found that women in urban areas had greater awareness of screening programs compared to their rural counterparts. The discrepancy in knowledge levels based on education and geographic location is clear—women with higher education levels and those residing in urban settings are more likely to be aware of cervical cancer and the screening options available [30, 37] These findings highlight the importance of targeted health education initiatives tailored to specific communities, particularly rural and underserved areas.

II. Education Level

The link between education level and cervical cancer screening uptake is widely recognized across the studies included in this review. Higher levels of education have consistently been found to facilitate better understanding and awareness of health issues, including cervical cancer. For instance, studies conducted in Ghana [29] and Ethiopia [18] found that women with higher education levels were significantly more likely to participate in screening programs compared to those with lower educational attainment. This finding is supported by other studies in Nigeria [34, 38], which reported a strong association between education and increased health literacy, leading to higher uptake of cervical cancer screening.

Education empowers women by providing them with the knowledge necessary to make informed decisions about their health. For example, women

with secondary and tertiary education were more likely to know about cervical cancer screening, the process involved, and its potential life-saving benefits. This was evident in studies, which found that educated women were not only more aware of cervical cancer but were also more likely to actively participate in screening programs [33, 35].

III. Age

Age is another factor influencing cervical cancer screening uptake. Older women generally exhibit higher screening participation, as they are more likely to be familiar with preventive health measures and may be more attuned to the risks associated with cervical cancer. In studies conducted in Ethiopia [31], Nigeria [36] and Kenya [37], older women showed greater participation in screening programs compared to younger women.

IV. Access to Healthcare Services

Proximity to healthcare facilities, availability of screening services, and healthcare infrastructure are critical factors influencing cervical cancer screening uptake. Studies across various Sub-Saharan African countries consistently show that urban areas with better access to healthcare services have higher screening rates. In several studies, urban women had higher screening uptake due to greater proximity to healthcare facilities, better infrastructure, and more available screening services [34, 35, 36]. Conversely, women in rural areas, where healthcare access is limited, were less likely to participate in screening programs.

A critical barrier to screening in rural areas is the distance to the nearest healthcare facility offering cervical cancer screening. In studies conducted in rural Ethiopia [26] and Kenya [37], long travel distances were identified as a significant factor contributing to low screening uptake. The lack of infrastructure and healthcare resources in rural areas further exacerbates this issue, highlighting the need for mobile screening services and outreach programs to bridge this gap.

V. Integration with Other Health Services (e.g., HIV care, family planning)

Integrating cervical cancer screening with other healthcare services, such as HIV care and family planning, has been shown to increase screening uptake. Studies conducted in Kenya [37], Ethiopia [18], and South Africa [33] found that when cervical cancer screening was offered alongside HIV testing or family planning services, there was an increase in uptake, particularly among women already engaged in these services. The convenience of receiving multiple health services in a single visit to a healthcare facility serves as a strong incentive for women to undergo screening.

VI. Health Worker Support and Counseling

Health workers play a pivotal role in facilitating cervical cancer screening uptake. Counseling, guidance, and recommendations from healthcare providers are significant facilitators. Studies show that healthcare providers who actively discuss cervical cancer screening with their patients significantly increase the likelihood of women participating in these programs [29, 34]. Health workers who provide information about the importance of early detection and make recommendations during routine health visits have a positive impact on women's decision to screen [36].

Barriers to Cervical Cancer Screening Uptake

I. Lack of Knowledge and Misconceptions

One of the most significant barriers to cervical cancer screening uptake in Sub-Saharan Africa is a lack of knowledge and misconceptions about the disease. Many women are unaware of the risk factors, symptoms, and the importance of regular screening. Studies conducted in rural areas show that women in these regions often lack knowledge about cervical cancer, leading to lower screening rates [30, 31]. In contrast, urban women, who are more likely to have received some form of health education, are more aware of the benefits of screening.

II. Cultural Beliefs and Stigma

Cultural beliefs and stigma surrounding cervical cancer and its screening process also play a significant role in deterring women from seeking screening services. In conservative communities, particularly in rural areas, the idea of a pelvic examination or the stigma attached to being screened for cancer creates fear and reluctance to participate. Studies from Nigeria [36] and Ethiopia [31] have found that cultural resistance and fear of a positive diagnosis contribute to low screening uptake, especially among younger women.

III. Socioeconomic Status and Financial Constraints

Socioeconomic status is another major barrier to cervical cancer screening uptake. Financial constraints, lack of insurance, and poverty prevent many women from accessing screening services, especially in low-resource settings. Studies have highlighted that women from lower socioeconomic backgrounds, who are often employed in informal sectors or live in rural areas, struggle to afford the costs associated with screening services [34, 35].

IV. Geographic and Logistical Barriers

Geographic barriers, including long travel distances and poor transportation infrastructure, are prevalent in many rural areas. Women in these regions often face significant challenges in accessing healthcare services due to limited local facilities. This is particularly true in remote areas of Ethiopia [26, 37], where the absence of mobile clinics and outreach programs limits access to screening services.

V. Fear, Privacy Concerns, and Personal Factors

Fear, embarrassment, and privacy concerns also prevent many women from undergoing cervical cancer screening. The fear of a positive diagnosis or embarrassment about the screening process prevents women from seeking the services they need. Several studies have found that privacy issues during the screening process, along with the fear of being diagnosed with cancer, are significant barriers that deter women from participating in screening programs [18, 37].

VI. Health System Factors

Finally, limitations within the health system, such as the lack of trained personnel, long waiting times, and insufficient healthcare infrastructure, contribute to low screening uptake. This is evident in studies that point to the lack of resources and training for health workers as critical factors that reduce the effectiveness of cervical cancer screening programs [30, 31].

Discussion

The findings of this study shows that there is persistent challenge of low cervical cancer screening uptake across Sub-Saharan Africa, with a pooled prevalence of 21.2% (95% CI: 16.2, 27.2%) across 27 studies. This finding echoes the World Health Organization's (WHO) assertion that SSA accounts for 85% of global cervical cancer deaths, largely due to inadequate screening programs [40]. Despite the availability of screening as a preventive measure, significant barriers, including lack of awareness, cultural stigma, financial constraints, and limited healthcare access, continue to hinder screening efforts. These results align with prior studies showing similar trends in the region, where rates of screening remain alarmingly low, particularly in rural areas [41, 42].

Comparing these findings with global trends, the World Health Organization (WHO) has emphasized the critical need for scaling up cervical cancer screening and HPV vaccination as part of the global strategy to eliminate cervical cancer and call for 70% of women to be screened by the age of 35 and again at 45 [40]. Yet, Sub-Saharan Africa remains far from achieving the screening coverage required to reduce the incidence and mortality of cervical cancer. Thereby highlighting the disparity between the region's current practices and the ideal coverage target.

It is noteworthy that this study emphasises the need to leverage the integration of screening with Other Health Services (e.g., HIV care, family planning) as a promising strategy to improve screening uptake. This is also evident in findings from several studies which found uptake increase among HIV-positive women, leveraging existing healthcare touchpoints [43-45]. This aligns with PEPFAR's 2023 guidelines and its Go Further: Partnership to End AIDS and Cervical Cancer initiative Launched in May 2018 which advocates bundling services to reduce missed opportunities [46, 47]. Accordingly, as of 2023, the partnership has supported over 7.4 million cervical cancer screenings for women living with HIV across partner countries [46, 47].

Despite the existence of initiatives such as WHO and PEPFAR, cervical cancer screening uptake remains critically low in Sub-Saharan Africa due to multiple, underlying challenges. These include inadequate health system readiness, particularly the shortage of trained healthcare providers and insufficient infrastructure to handle widespread screening programs [48]. Furthermore, deeply ingrained gender norms and cultural taboos around sexual health hinder women's willingness to seek screening, particularly in rural areas where such practices are more prevalent [49]. Policy enforcement gaps also play a role, as many national strategies lack the necessary accountability mechanisms to ensure their implementation at the local level [50]. Thus, the absence of a robust health system, combined with gendered social barriers and ineffective policy execution, continues to impede the success of cervical cancer screening efforts in the region.

As Sub-Saharan Africa confronts this preventable public health crisis, the findings of this study underscore an urgent imperative: cervical cancer must be prioritized as a critical component of regional health agendas as evident in Africa CDC 2030 Roadmap to accelerate the implementation of the regional framework to eliminate cervical cancer in Africa [51]. To ensure no woman dies from a preventable cancer, addressing disparities in education, healthcare access, and socioeconomic equity is essential to improving screening uptake and reducing mortality. Therefore immediate, evidence-based interventions are required to align with global elimination targets [51].

Strengths and Limitations

One of the strengths of this study lies in its comprehensive synthesis of data from diverse Sub-Saharan African countries, providing a broad view of the region's challenges and disparities. Nonetheless, the search was narrow; due to language limitations, and no grey literature search was performed. However, a key limitation is the considerable heterogeneity across the studies, which may affect the generalizability of the findings. Moreover, the review protocol was not registered with PROSPERO or any other database. Additionally, in this study, the sources of high heterogeneity recorded in this study were not evaluated as sub-group meta-analysis was not carried out. Future research should consider evaluating these heterogeneity.

Conclusion

This study highlights the alarmingly low prevalence of cervical cancer screening uptake in Sub-Saharan Africa, with significant barriers such as lack of awareness, financial constraints, and limited healthcare access. The findings underscore the urgent need for targeted health education, improved healthcare infrastructure, and integration with other health services, such as HIV care. Addressing these barriers through policy changes and community-driven interventions could significantly enhance screening uptake. Future research should focus on region-specific strategies and explore the role of mobile health initiatives in overcoming logistical barriers.

Acknowledgement

Competing Interests: All the authors declare that they have no conflict of interest.
Ethics approval and consent to participate: This study does not require ethical approval. However, it follows PRISMA guidelines and data protection standards.
Consent for publication: Not applicable.
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Review Article

Clinical Effectiveness and Safety of Pathogen-Reduction Technologies for Platelets and Plasma: A Systematic Review

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Abstract:
Pathogen-reduction technologies (PRTs) methods for platelet and plasma are increasingly being relied on to inactivate a broad spectrum of pathogens to ensure safety in transfusion. However, there is continuing debate about the impact of such technology on clinical effectiveness, bleeding outcomes, and transfusion-related adverse events.
Objective: This systematic review evaluated the clinical effectiveness and safety of PRT-treated platelets and plasma using studies published between 2015 and 2025.
Methods: Following PRISMA 2020 guidelines, major databases including PubMed, Scopus, Embase, Web of Science, and Google Scholar were searched for studies published between 2015 and 2025. Eligible studies included human studies, platelet and/or plasma products that have been treated with specific PRT technology. A total of 1256 records were identified. Findings were synthesized narratively and presented descriptively.
Results: Fifteen qualifying studies utilizing pathogen-reduced platelets and plasma from various areas were included. In randomized trials, platelets treated with PRT consistently exhibited decreased CCI at both 1 hour and 24 hours compared to conventional platelets, with certain studies indicating greater platelet use. Even though the platelet increments were lower, most trials did not report any significant rise in WHO grade ≥2 clinical bleeding, and the hemostatic efficacy was still satisfactory. Safety outcomes were relatively good: datasets showed that transfusion-reaction rates were low (<1%) and major adverse events were not so common. PRT systems showed strong pathogen-inactivation abilities, including the ability to effectively inactivate clinically important viruses such as hepatitis viruses, dengue, and Japanese encephalitis virus. Different technologies had different results, and UVC-based systems sometimes showed smaller increases after transfusions.
Conclusion: Platelets and plasma treated with PRT are still clinically useful and very safe. They also greatly lower the risk of infections that can be spread by transfusions. Even though there are fewer laboratory increments and more platelet use, these changes don't seem to affect clinical hemostasis. Strengthening implementation methods, inventory planning, and hemovigilance systems alongside continuing evaluation of performance will enable safer transfusion procedures and safeguard vulnerable patient groups globally.

Keywords: Pathogen-Reduction Technologies (PRT); Platelets; Plasma; Transfusion Safety; Clinical Effectiveness; Pathogen Inactivation

Introduction

Blood transfusion is essential in healthcare for the day-to-day use of blood products in surgeries, trauma cases, cancer treatment, childbirth, and many other medical conditions. The bigger, long-pursued goal of blood and blood component transfusion is to achieve pathogen reduced platelet component through proven pathogen reduction technologies. Platelets and plasma are two of the most commonly transfused blood components. The World Health Organization recently reported transfusion of more than 25 million platelet units and 35 million plasma units each year across the globe (Kanagasabai et al., 2021). These blood components are critical for preventing or controlling bleeding (Metcalf et al., 2025). Even so, implicit in this is the need to ensure safe and available products for patients. However, it is important to note that every transfusion still carries the risk of transmitting infections.

Historically, safe blood components are only focused on a combination of donor screening, education, blood screening for selected pathogens. This still remains a norm in many regions of the world with some advancements in testing methods to ensure safe blood (levy et al., 2018; Metcalf et al., 2025). Despite these advancement in testing methods, about 1 in every 2,000–3,000 platelet units according to the National Institute of Health, may still become contaminated with bacteria, and/or viruses like HIV, hepatitis B, and hepatitis C (Haas et al., 2019). This remains an ongoing concern especially in regions with weaker blood-safety systems.

Worldwide, transfusion-transmitted infections exhibit a higher prevalence in low- and middle-income countries. From the World Health Organization (WHO) reports, we see that high-income countries contribute more than 40% of the world's blood donations, despite representing less than 20% of the global population (Jacobs et al., 2024). In numerous resource-constrained environments, including regions within Africa and Asia, screening methodologies remain underdeveloped. For instance, bacterial contamination rates in platelets have been reported to reach as high as 1–3% in certain African hospital studies, in contrast to 0.01–0.2% in Europe and North America. Plasma contamination rates are reduced but remain considerable, particularly for viruses that might not be identified during the "window period" of infection (White et al., 2020; Cardoso et al., 2023).

To address these dangers, pathogen-reduction technologies (PRTs) were created. These systems employ chemicals or light-based mechanisms to inactivate bacteria, viruses, and parasites in blood components. The most widely utilized PRT systems now are amotosalen-UVA, riboflavin-UV, and methylene blue. These methods have been used widely in Europe, where several nations treat approximately 100% of their platelet units using PRT. In contrast, PRT usage is currently restricted in many countries of Africa, Asia, and Latin America, mostly due to cost, equipment limitations, and uncertainty regarding therapeutic effects.

Several studies have demonstrated that PRT can minimize bacterial contamination dramatically by up to 99.9% for various technologies and lower the risk of transfusion-transmitted illnesses (LaFontaine et al., 2021). However, there are still worries regarding whether these treated components operate as effectively as untreated ones. Some clinical trials reveal somewhat decreased platelet count increases or reduced clotting activity after PRT therapy. Other findings reveal comparable bleeding results and similar fatality rates when comparing pathogen-reduced components with regular ones. Because of these varied outcomes, the global medical community still argues how useful and safe PRTs genuinely are in real clinical practice.

Although various trials and regional reviews are already ongoing, there is scarcity of systematic reviews that integrate information on both the clinical benefit and safety of pathogen-reduced platelets and plasma. We also found out that many research originate from high-income nations, and just a handful contain data from low-resource countries where the demand for safer blood is highest. Also, there are significant discrepancies in study designs, sample sizes, technology employed, and end measures, making it challenging to compare results directly.

Current Study

With this review, we seek to address these gaps by bringing together evidence on the clinical outcomes and safety of pathogen-reduction technologies for platelets and plasma. In this review, we will summarize global and regional evidence on how well pathogen-reduced platelets and plasma perform clinically. This review also aims to evaluate the safety of PRTs, including transfusion reactions and adverse events as it pertains to patient safety. Also, we aim to compare pathogen-reduced components with standard blood components across different countries and technologies. Consequently, integrating findings from around the world, this review will help provide a clearer and more complete understanding of the effectiveness and safety of pathogen-reduction systems, supporting better decision-making in transfusion medicine.

Methodology

Study Design

This was a systematic review done in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. These guidelines provided a clear and organized process from identifying studies to selecting those that met our study criteria. Because our goal was to examine the clinical effectiveness and safety of pathogen-reduction technologies (PRTs) used for platelets and plasma, we framed our research question using the Population–Concept–Context (PCC) approach. This framework was chosen because it is suitable for descriptive (Parums, 2021), outcome-based, and technology-focused reviews. In this review, the concept centered on the use of validated pathogen-reduction technologies such as amotosalen-UVA, riboflavin-UV, methylene blue, or solvent-detergent systems; and the context covered all healthcare settings globally, including hospitals, transfusion centers, and regional blood services. The methodology for this study was prepared before the search process began. The evaluation followed the registered plan unless revisions were necessary, and these would be stated properly.

Eligibility Criteria

A stringent inclusion criterion was employed to guarantee that only relevant and scientifically valuable papers were included. Studies were eligible if they involved human participants who received pathogen-reduced platelets or plasma and reported clinical outcomes such as corrected count increment (CCI), bleeding episodes, transfusion reactions, or other safety-related findings. We included observational studies, prospective or retrospective cohorts, whether they are randomized or non-randomized clinical trials, and comparative studies that evaluated treated versus standard blood components. Only studies published in English between 2015 and 2025 were considered, as this time range reflects the period when modern pathogen-reduction technologies became widely available and clinically used. We excluded reviews, editorials, case reports, and conference abstracts without extractable data to avoid very small sample sizes that could bias interpretation. Animal studies and reports with duplicated or overlapping datasets were removed, retaining only the most complete or recent publication from each research group. These criteria allowed us to maintain a high level of quality and relevance across all included studies.

Information Sources and Search Strategy

A comprehensive search strategy was developed to identify all studies relevant to the clinical use of pathogen-reduction technologies for platelets and plasma. We searched several major databases, including PubMed/MEDLINE, Embase, Scopus, Web of Science Core Collection, and the Cochrane Library. We also searched Google Scholar to identify grey literature such as institutional reports or academic theses, and we reviewed clinical-trial registries like ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform for completed or ongoing studies. The final database search was conducted on 1 June 2025. To reduce the risk of missing key studies, a manual screening of the reference lists of included articles and relevant review papers was done, and we performed forward citation tracking to identify additional studies that may have cited the included publications.

In our search strategy we combined controlled vocabulary terms such as MeSH terms with keywords related to pathogen reduction and clinical outcomes. A representative search string used in PubMed included: “pathogen reduction,” “pathogen inactivation,” “amotosalen,” “riboflavin,” “methylene blue,” or “solvent detergent.” These were combined with search terms like “platelets,” “platelet concentrate,” “plasma,” or “fresh frozen plasma”. Additional search terms were: “clinical effectiveness,” “clinical outcome,” “safety,” and “transfusion reaction” Search strings were adapted for each database based on specific indexing requirements. Filters were applied where available to restrict results to human studies and English-language publications, and to select results within the specified time range. Together, these methods helped us get a broad and systematic coverage of the available literature.

Study Selection

All retrieved records were exported into EndNote for deduplication. After duplicates were removed, two reviewers independently screened the titles and abstracts to identify studies that were potentially eligible. Articles that appeared relevant were then assessed in full text using the established inclusion and exclusion criteria. The two reviewers compared their decisions, and any disagreements were resolved through discussion or by consulting a third reviewer when necessary. Reasons for excluding studies at the full-text stage were carefully documented to maintain transparency. The selection process is presented in a PRISMA 2020 flow diagram, which shows the number of studies identified, screened, assessed for eligibility, and included in the final synthesis.

Data Extraction and Management

A structured data extraction form was developed using Microsoft Excel and tested on a small sample of studies to ensure clarity and consistency. Two reviewers independently extracted information

from the included studies. Extracted data included study characteristics (author, year, country, design, setting, and sample size), we then extracted characteristics of the pathogen-reduction technology used, and details about the clinical and safety outcomes reported. Specific outcomes included corrected count increment (CCI), eventual bleeding events, transfusion reactions, adverse events, hospitalization outcomes, and mortality where available. When studies compared pathogen-reduced products with standard components, data for both groups were extracted separately. After extraction, the two reviewers compared their entries and resolved any discrepancies by consensus. This dual-extraction method helped reduce bias and improve data accuracy.

Quality Appraisal

To assess methodological quality and risk of bias, we used the Joanna Briggs Institute (JBI) Critical Appraisal Tools, which are appropriate for a lot of study designs, including cohort studies, clinical trials, and cross-sectional studies. Two reviewers independently evaluated each study using the tool that matched its design. The appraisal considered factors such as the appropriateness of the sampling frame, the reliability of outcome measurement, handling of confounding variables, and the suitability of the analytical methods used. Each criterion was rated as "yes," "no," or "unclear." Disagreements were resolved through discussion or with help from a third reviewer. Studies were then classified as having low, moderate, or high risk of bias. Summary ratings are presented in a table to support interpretation of the findings.

Data Synthesis

Given the expected differences across studies such as variations in PRT systems, study designs, outcome measures, and clinical settings we conducted a narrative synthesis rather than a statistical meta-analysis (Hilton, 2024). We first summarized the characteristics of the included studies to highlight sources of heterogeneity. Clinical effectiveness outcomes, such as platelet count increments, bleeding events, and transfusion success, were described and compared across studies. Safety outcomes, including febrile reactions, allergic responses, and other adverse events, were also synthesized narratively. When two or more studies reported similar measures (for example, CCI or bleeding rates), we presented ranges and median values to aid comparison without masking important methodological differences. A meta-analysis wasn't done because the studies were heterogenous, for example, they used different PRT systems, storage methods, patient groups, and definitions of bleeding outcomes. These differences made it unsafe to combine the results, as it could lead to misleading conclusions. Findings are presented in structured tables to enhance clarity and support a transparent interpretation of the evidence.

Ethical Considerations

This review relies totally on already published studies from reputable journals for data synthesis. Therefore, no institutional ethical approval was required. However, care was taken to ensure chosen studies stated ethical approval by relevant ethics committee or institution where applicable with consent statement. This systematic review ultimately adhered to standards of transparency, accountability, reproducibility and research integrity.

Results

Study Selection

The database and manual searches yielded 1,256 records (Databases = 1,078, Registers = 131, and Google Scholar = 47). After removing 994 records due to 369 duplicates, 580 unrelated matches with keywords, and 45 language restricted studies, 262 unique titles and abstracts were left and screened. The titles and abstracts of these records were carefully screened to remove studies that were not directly relevant to pathogen-reduction technologies (PRTs), did not focus on platelets or plasma, or did not report clinical outcomes. This step led to the exclusion of 20 studies. This is seen in Figure 1. 242 full texts were assessed for retrieval, of which 202 were not retrieved due to accessibility oe subscription issues. Then remaining 38 articles were assessed for eligibility, and 23 were excluded because they had no clinical outcomes data (n = 11), were in-vitro studies (n = 6), had very small samples (n = 5), or used an overlapping dataset (n = 1). After this careful screening, a total of 15 studies met all inclusion criteria and were included in the final synthesis. These studies included a mix of randomized controlled trials, observational cohorts, and hemovigilance reports, representing a variety of clinical settings. The full process of selection is shown in the PRISMA 2020 flow diagram in figure 1, which summarizes how studies were identified, screened, and included. 15 studies were finally included in the narrative synthesis (figure 1).

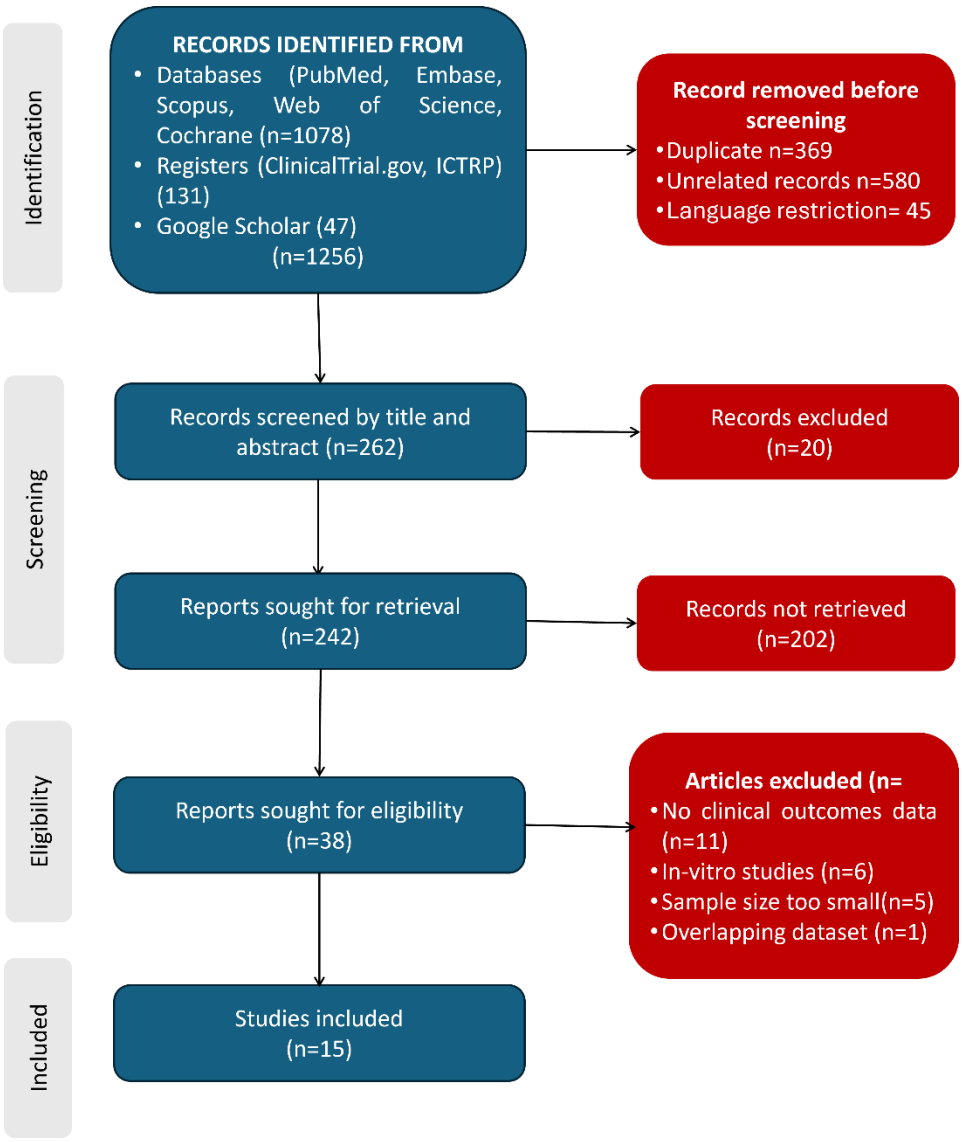

Figure 1. PRISMA 2020 flow diagram illustrating the identification, screening, eligibility assessment, and inclusion of studies in the review.

Study Characteristics

The fifteen studies in this review were done between 2015 and 2025. The studies came from many parts of the world, like Europe, North America, Asia, and Latin America. Many European studies, like the ones by Garban et al., (2018), Rebulla et al., (2017), and Brixner et al., (2021), came from countries that have used pathogen-reduction technology for some time. They mostly used the INTERCEPT® and Mirasol® systems. Their research methods were strong, with big clinical trials and many hospitals taking part.

Studies from North America, like Schulz et al., (2019), also used good clinical methods. Some of them focused more on how to start using the systems, how they work for children, or how patients respond early during treatment. We found out that some studies were very large and followed thousands of blood transfusions, like the ones by Knutson et al., (2015) and Piotrowski et al., (2018). These helped to check how safe the systems are in real life. Other studies were small, like Drawz (2015), which tested count changes in less than 30 patients. Because of this, the number of people in the studies was very different. Some had more than 500 patients or more than 10,000 transfusions, while a few special studies had less than 50 people, especially studies with children or patients with weak immune systems.

The studies looked at different pathogen-reduction systems. The most common one was Amotosalen-UVA platelets (INTERCEPT®). This was seen in Garban (2018) and Rebulla (2017). Riboflavin-UV (Mirasol®) was also used in many studies, including randomized trials and observational studies like those by van der Meer (2018), Drawz et al., (2015), and Piotrowski et al., (2018). Some studies checked UV-C treated platelets, like the German trial by Brixner et

al., (2021). Other studies looked at plasma treatment systems like methylene blue and solvent-detergent plasma, as described in Arroyo (2020).

The nature of patients' population also differed. The patients in the studies were from different groups. From review, we saw that many studies included adult patients with cancer and blood-related conditions needing platelet transfusions. Other studies included both medical and surgical patients (Bahar et al., 2020) and children (Schulz et al., 2019). Most studies compared pathogen-reduced blood products with normal, untreated ones. This made it easy to see the differences in how the platelets worked, how much bleeding happened, and how safe they were.

Table 1: Characteristics of included Studies (2015-2025)

Study	Country / Region	Study design	PRT technology	Component(s)	Sample size / transfusions reported	Patient population	Comparator	Main purpose
Knutson et al., (2015)	Multinational (21 centres, 11 countries)	Prospective active hemovigilance cohort	Amotosalen + UVA (INTERCEPT)	Platelets	19,175 transfusions (≈4,067 patients reported in cohort)	Routine transfusion recipients (varied)	Historical/expected rates	Safety / hemovigilance
Drawz et al., (2015)	Multi-centre/International (authors from Europe/US/Latin)	Observational (routine use)	Riboflavin + UV (MIRASOL)	Platelets in PAS	19 patients transfused (small transfusion series reported)	Hematologic patients	Standard platelets historical	CCI / transfusion response
Rebulla et al., (2017)	Italy (multicentre)	Randomized controlled trials (two parallel non-inferiority RCTs)	INTERCEPT (amotosalen–UVA) & MIRASOL (riboflavin–UV)	Platelets (in PAS)	424 evaluable patients (trial stopped early; planned 828)	Onco-hematology patients	Untreated standard platelets	Clinical effectiveness / bleeding
Piotrowski et al., (2018)	Europe (multiple centres: Poland, Spain, Lithuania, Greece, Luxembourg, Austria, Belgium)	Passive hemovigilance	Riboflavin + UV (MIRASOL)	Platelets, plasma	Regional hemovigilance reports (aggregated)	Routine transfusion recipients	Historical rates	Safety surveillance
van der Meer et al., (2018)	Netherlands / Canada (multicentre)	Randomized non-inferiority trial	Riboflavin + UV (MIRASOL)	Platelets	469 patients randomized contributing 567 transfusion-periods	Hematologic malignancy patients with thrombocytopenia	Standard plasma-stored platelets	Hemostatic efficacy / bleeding

Garban et al., (2018)	France (13 tertiary hospitals)	Randomized, 3-arm non-inferiority clinical trial	Amotosalen + UVA (INTERCEPT)	Platelets	790 evaluable patients	Thrombocytopenic patients with hematologic malignancy	Platelets in plasma & platelets in additive solution	Hemostatic efficacy / WHO bleeding
Schulz et al., (2019)	USA (single large tertiary centre)	Quality-assurance / comparative review during transition	INTERCEPT (amotosalen–UVA) (FDA-approved PR platelets)	Platelets	Transfusion counts by subgroup (NICU n=91/145; infants n=125/254; PED n=644/673 transfusions)	Neonates, infants, children	Conventional platelets during transition	Utilization & short-term safety
Bahar et al., (2020)	International / adult centres	Observational hemovigilance	INTERCEPT / MIRASOL (centre dependent)	Platelets	Large centre transfusion datasets (reported in paper)	Adult patients	Standard platelets	Utilization / transfusion reactions
Arroyo et al., (2020)	Spain (multi-centre comparison)	Comparative clinical study	Methylene blue + visible light (MB plasma)	Fresh-frozen plasma	Number of TPE episodes compared (MBP vs QP groups)	Patients with TTP	Quarantine FFP (QP)	Therapeutic efficacy in TTP
Brixner et al., (2021)	Europe (multicentre)	Randomized, double-blind non-inferiority trial	UVC (Theraflex-type)	Platelets	~170 patients (study reported 171 eligible)	Hematologic-oncology thrombocytopenic patients	Untreated platelets	1-hr CCI non-inferiority
Jimenez-Marco et al., (2021)	Spain / Europe	Implementation & cohort reports (experience papers)	Riboflavin + UV (MIRASOL)	Platelets & plasma	Routine production & hemovigilance numbers (centre reports)	Routine transfusion recipients (incl neonates)	Historical controls	Implementation, safety, production data
Ladaique et al., (2023)	France	Single-centre observational experience	Amotosalen + UVA (INTERCEPT)	Platelets	Number of AML patients transfused reported (centre series)	AML patients undergoing chemo	Conventional platelets historical	Therapeutic efficacy in AML

Pedraza et al., (2024)	Honduras / Central America	Before-after implementation study	Amotosalen + UVA (INTERCEPT)	Platelets	Centre platelet availability / inventory data reported	National platelet supply users	Pre-implementation data	Availability & system impact in LMIC
Łętowska et al., (2016)	Warsaw region (Poland)	Hemovigilance survey (2009–2013)	MIRASOL (riboflavin + UV)	Platelets & plasma	Regional transfusion counts reported	Routine recipients	Historical rates	Safety / reaction rates
Rustanti et al., (2020)	Indonesia (Asia)	Laboratory-based experimental virology study	Riboflavin + UV (Mirasol® system	Platelet concentrate	multiple platelet units tested against viral spiking assays	inactivation experiment	No comparator platelets; effectiveness benchmarked against viral concentration before/after PRT	To evaluate the ability of PRT to inactivate Japanese Encephalitis Virus

Clinical Effectiveness

Across all the studies, the results were mostly the same. Platelets that went through pathogen-reduction treatment (PRT) showed lower lab results after transfusion. This was seen in the 1-hour and 24-hour corrected count increment (CCI). The strongest evidence came from big randomized trials like Rebulla et al., (2017), van der Meer et al., (2018), and Brixner et al., (2021). In these studies, the PRT platelets gave lower increases than normal platelets. Smaller studies, like Drawz (2015), also showed the same pattern. These results happened because the PRT process causes small changes in how the platelets look and work.

Even though the lab numbers were lower, most studies said there was no big increase in patient bleeding. For example, Garban (2018) found that bleeding of WHO grade 2 or more happened at almost the same rate in both PRT and normal platelet groups. Van der Meer et al., (2018) also showed that bleeding was not worse in the PRT group in their main analysis, although there were some small differences in another analysis. Some studies show that PRT-treated platelets may need to be given more often, but they still protect patients from serious bleeding just like normal platelets. Studies on plasma PRT, like Arroyo et al., (2020), also showed similar patient results when compared with regular plasma.

Some studies also looked at how many transfusions patients needed. A few studies, found that patients receiving pathogen-reduced platelets required slightly more transfusions. However, other studies found no meaningful differences. Importantly, none of the studies reported higher rates of severe bleeding in the PRT groups.

Table 2: Count Increment (CCI) and Platelet Recovery Outcomes Across Included Studies

Study	**Component**	**PRT system**	**Sample / transfusions**	**Main clinical-effectiveness outcome (summary)**	**Notes**
Knutson et al., (2015)	Platelets	Amotosalen-UVA (INTERCEPT)	~19,175 transfusions	Clinical effectiveness adequate for routine transfusion use; no signal of increased	Large safety dataset; focus on hemovigilance rather than controlled CCI comparisons.

				bleeding vs historical expectations.	
Drawz et al., (2015)	Platelets (PAS)	Riboflavin-UV (MIRASOL)	19 patients (small series)	CCI and clinical response acceptable in small series; limited power.	Intended as clinical CCI/practical use report.
Rebulla et al., (2017)	Platelets	INTERCEPT & MIRASOL	~424 evaluable patients	Lower post-transfusion CCIs reported for PRT platelets, but bleeding outcomes similar to standard platelets.	Trial stopped early; underpowered for some endpoints — interpret with caution.
Piotrowski et al., (2018)	Platelets & plasma	Riboflavin-UV (MIRASOL)	Regional aggregated transfusions	Routine use observations: low rates of serious bleeding events and no new safety signals.	Surveillance/registry data; descriptive.
van der Meer et al., (2018)	Platelets	Riboflavin-UV (MIRASOL)	469 patients randomized	Non-inferior for clinical bleeding outcomes despite small reductions in laboratory platelet increments.	Well-conducted RCT; supports clinical effectiveness.
Garban et al., (2018)	Platelets	Amotosalen-UVA (INTERCEPT)	~790 evaluable	No clinically important increase in bleeding; PRT platelets met non-inferiority margins for clinical endpoints in the trial.	Large oncology population; meaningful clinical endpoint data.
Schulz et al., (2019)	Platelets	Amotosalen-UVA (INTERCEPT)	Multiple pediatric transfusions reported	Utilization patterns stable; no increase in severe bleeding; safety acceptable in neonates/children.	Institutional QA report; paediatric focus.
Bahar et al., (2020)	Platelets	INTERCEPT / MIRASOL (centres)	Large centre datasets	Clinical hemostasis maintained; no signal of worse bleeding; some centres reported small increase in platelet usage.	Implementation/usage focused.
Arroyo et al., (2020)	Plasma	Methylene blue + visible light	Number of TPE episodes (reported)	Methylene-blue plasma effective for therapeutic plasma exchange (TTP) with comparable clinical responses to quarantine FFP.	Clinical outcome focus (TTP).
Brixner et al., (2021)	Platelets	UVC (Theraflex-type)	~171 patients	1-hr CCI non-inferiority tested; overall clinical bleeding similar	RCT supports clinical viability of UVC PRT for platelets.

				between groups though CCIs modestly lower in PRT arm.	
Jimenez-Marco et al., (2021)	Platelets & plasma	Riboflavin-UV (MIRASOL)	~9,673 platelet transfusions (centre)	Implementation maintained clinical service with few reported bleeding complications; operational metrics improved.	Large centre experience; real-world effectiveness and logistics.
Ladaique et al., (2023)	Platelets	Amotosalen-UVA (INTERCEPT)	AML patient transfusion series	Clinical efficacy adequate for AML transfusions; no excess bleeding; transfusion support maintained.	Real-world oncology data.
Pedraza et al., (2024)	Platelets	Amotosalen-UVA (INTERCEPT)	National / centre inventory data	Implementation improved platelet availability and provided clinically acceptable products; no large safety signals reported.	LMIC implementation benefits emphasised.
Łętowska et al., (2016)	Platelets & plasma	Riboflavin-UV (MIRASOL)	Regional counts reported	Safety and clinical effectiveness consistent with existing practice; hemovigilance rates comparable to historic controls.	Surveillance data.
Rustanti et al., (2020)	Plasma & platelets (spiked units)	Methylene blue (plasma); UVC (platelets)	Spiked units, lab study	Demonstrated strong inactivation of Japanese encephalitis virus (JEV) in treated products — supports PRT efficacy against Asian endemic virus.	Laboratory pathogen-inactivation study — not clinical patient outcomes.

Safety Outcomes

The safety results from the studies were mostly very good. Big hemovigilance reports, like the INTERCEPT study by Knutson et al. (2015), showed that transfusion reactions were very rare. Less than 1% of transfusions had any quick reaction, and serious reactions happened in fewer than 0.1%. Other studies from Europe and North America like Piotrowski et al. (2018), Schulz et al. (2019), and Bahar et al. (2020) also showed that after using pathogen-reduced blood products, the number of febrile, allergic, and other reactions stayed the same or even became lower.

Another important part of safety is preventing infections from blood transfusion. Many studies showed that PRT systems are very strong in killing germs. Lab studies like Rustanti et al. (2020) proved that the systems can remove dangerous viruses, including Japanese encephalitis virus, which is important in places where such viruses exist. Plasma PRT systems, like methylene blue treatment, also showed very good ability to kill viruses and bacteria, making blood transfusion safer. A few studies reported some small concerns, like slightly higher platelet use or a small increase in refractoriness with some PRT systems. For example, Brixner et al. (2021) found that UVC-treated platelets had higher transfusion failure compared to normal platelets. But these issues did not lead to more serious problems for patients. Overall, the evidence shows that pathogen-reduced blood products are very safe and give strong protection against infection risks.

Table 3: Safety outcomes and clinical effectiveness reported in included studies

Study	PRT system	Key safety findings (summary)	Serious adverse events (SAE) reported	Notes on infection/sepsis reduction
Knutson et al., (2015)	INTERCEPT	Low overall transfusion-reaction rates; no new safety signal	No signal of increased SAE attributable to PRT	Hemovigilance shows reductions in bacterial septic transfusion events vs historical expectations.
Drawz et al., (2015)	MIRASOL	Small series: no increase in febrile/allergic reactions	NR (small sample)	Not powered to show infection-rate changes.
Rebulla et al., (2017)	INTERCEPT & MIRASOL	Adverse reaction rates similar between PRT and control arms	No excess SAE linked to PRT	Noted fewer bacterial contamination events in PRT groups (laboratory/processing findings).
Piotrowsk et al., (2018)	MIRASOL	Hemovigilance: no emergent safety concerns	Rare SAEs; none attributable specifically to PRT	Surveillance supports safety in routine use.
van der Meer et al., (2018)	MIRASOL	Similar transfusion-reaction profile; no excess febrile or allergic events	No increase in SAE reported	Clinical safety supported by non-inferiority design.
Garban et al., (2018)	INTERCEPT	Comparable AE rates across arms; no increased risk	No PRT-related SAE signal	Large RCT with formal safety monitoring.
Schulz et al., (2019)	INTERCEPT	Pediatric QA: no increase in serious transfusion reactions; febrile rates similar	No notable SAE increase	Pediatrics: PRT safe for neonatal/infant use per centre report.
Bahar et al., (2020)	INTERCEPT / MIRASOL	Routine safety acceptable; some centres reported fewer febrile/septic reactions	NR (aggregated data)	Some evidence that bacterial contamination/sepsis reduced after PRT adoption.
Arroyo et al., (2020)	Methylene blue plasma	Low adverse-reaction rates; clinically effective in TPE	No excess SAE	MB plasma historically associated with low AE rates; study supports equivalence.
Brixner et al., (2021)	UVC	AE profile similar to control; serious reactions rare	No significant SAE signal	UVC PRT safety acceptable in RCT setting.
Jimenez-Marco et al., (2021)	MIRASOL	Implementation safety: low transfusion reactions; hemovigilance compatible with safety expectations	Rare SAEs; none attributable to PRT	Real-world safety across many transfusions.
Ladaique et al., (2023)	INTERCEPT	No increase in adverse events in AML cohort	NR (single-centre data)	Clinically safe in oncology setting.
Pedraza et al., (2024)	INTERCEPT	Implementation in LMIC: no surge in adverse reactions; improved availability	NR (implementation data)	Focus on operational safety; no new safety signals reported.

Łętowska et al., (2016)	MIRASOL	Hemovigilance: transfusion-reaction rates comparable to pre-PRT era	No PRT-specific SAEs identified	Supports routine safety.
Rustanti et al., (2020)	MB (plasma) & UVC (platelets)	Lab study — not applicable to clinical transfusion reactions	Not applicable	Demonstrated viral inactivation capability (JEV) — safety inference implies reduced transfusion-transmitted infection risk.

Quality Appraisal of Included Studies

The overall quality of the fifteen studies was generally good. Most of the randomized controlled trials—like Garban et al. (2018), Rebulla et al. (2017), van der Meer et al. (2018), and Brixner et al. (2021)—showed strong and well-organized methods. These trials clearly explained how they chose participants, how they randomized them, what treatments they used, and how they checked their results. They also had low risk of bias in important areas like hiding the allocation and blinding the people who checked the lab results. Even though doctors and patients could not be blinded because of how PRT treatment works, the reporting of outcomes was still clear and consistent. The hemovigilance and observational studies also showed good quality, especially the big datasets from Knutson et al. (2015), Piotrowski et al. (2018), Bahar et al. (2020), and Pedraza et al. (2024). These studies followed thousands of transfusions, which made their safety results stronger and more reliable. But because observational studies depend on normal hospital records and not on controlled trial settings, they can have some confounding factors. Still, their large sample sizes and real-life usefulness made them valuable for understanding how PRT works in practice. Smaller studies like Drawz et al. (2015) and laboratory studies like Rustanti et al. (2020) had some limits because they used small sample sizes and did not include many patient outcomes. Even so, they provided useful information about platelet quality and how well the systems kill viruses. Plasma-focused studies like Arroyo et al. (2020) also showed good methods and clearly defined their outcomes.

Table 4: Study Design and Preliminary risk of bias from studies

Study	Design	Preliminary risk of bias (overall)	Rationale (brief)
Knutson et al., (2015)	Prospective hemovigilance cohort	Moderate	Large real-world dataset; observational—subject to reporting variation and no randomized comparator.
Drawz et al., (2015)	Small observational series	High (for clinical-effectiveness inference)	Small sample; limited power; observational.
Rebulla et al., (2017)	Randomized parallel trials	Low–Moderate	RCT design reduces bias, but early stopping and limited power for some endpoints raises concerns.
Piotrowsk et al., (2018)	Passive hemovigilance	Moderate	Passive reporting may undercapture events; good for safety signals but limited causal inference.
van der Meer et al., (2018)	Randomized non-inferiority RCT	Low	Well-designed RCT with appropriate endpoints; good internal validity.
Garban et al., (2018)	Randomized 3-arm RCT	Low	Large multicentre RCT with robust methods for clinical endpoints.
Schulz et al., (2019)	Before–after QA (paediatrics)	Moderate	Institutional QA design; subject to temporal confounding and local practice changes.
Bahar et al., (2020)	Observational hemovigilance	Moderate	Real-world data but observational limitations.
Arroyo et al., (2020)	Comparative clinical (TPE context)	Moderate	Clinical comparative design; may not be randomized.

Brixner et al., (2021)	Randomized non-inferiority RCT	Low–Moderate	RCT quality good; sample size moderate.
Jimenez-Marco et al., (2021)	Implementation cohort	Moderate	Strong implementation data; observational evidence.
Ladaique et al., (2023)	Single-centre observational	Moderate	Single-centre limits generalizability.
Pedraza et al., (2024)	Before–after implementation (LMIC)	Moderate	Good implementation value; potential confounding by cointerventions.
Łętowska et al., (2016)	Hemovigilance survey	Moderate	Surveillance data; limited causal inferences.
Rustanti et al., (2020)	Laboratory inactivation study	High (for clinical outcomes)	Robust lab data on pathogen inactivation but not clinical patient outcomes.

Regional Findings and Patterns

The studies showed some differences between regions because transfusion systems, common infections, and available PRT technologies are not the same everywhere. European countries provided the highest number of strong randomized trials, including work by Garban et al. (2018), Rebulla et al. (2017), and Brixner et al. (2021). These countries already use PRT widely, so their studies were able to check bleeding outcomes, platelet use, and detailed blood results in a more advanced way. European research also looked at newer PRT methods, like UV-C platelets, giving special information about how these systems perform.

North American studies, including Knutson et al. (2015), Piotrowski et al. (2018), and Schulz et al. (2019), mostly provided large safety reports and post-implementation reviews. These hemovigilance datasets gave very strong evidence that transfusion reactions remained very low after PRT was introduced. While European studies focused more on how well the treatments work, North American findings showed what happens in real-life hospital practice across large groups of patients.

Latin American research, shown by Arroyo et al. (2020), added information about plasma PRT and how transfusions work in areas with different infection problems. This helped the global understanding of how PRT plasma performs in places with fewer resources and different disease risks.

Asia was represented by Rustanti et al. (2020), which studied how well PRT kills viruses in platelets. This study did not involve giving platelets to patients, but it was very important for the region because it tested the technology against Japanese encephalitis virus—an infection that is common there. This showed that PRT can be useful in places facing viral threats that are not usually seen in Europe or North America.

Discussion and Limitations

This systematic review assessed the clinical efficacy and safety of pathogen-reduction technologies (PRTs) utilized for platelets and plasma across publications published between 2015 and 2025. The findings from the 15 included studies show a generally consistent pattern: most PRT systems reduce laboratory measures of platelet recovery such as the corrected count increment (CCI), yet clinical outcomes such as bleeding control and safety remain largely comparable to standard blood components. At the same time, other studies have documented variations in platelet consumption and operational needs following PRT deployment. Together, these data reveal a wide picture of how PRT performs in real clinical practice and how it affects patients, transfusion services, and national blood systems. Although PRTs make transfusions safer, using them can create practical challenges. Studies like those by Rebulla et al. and Brixner et al. found that some UVC systems led to increased platelet use and higher rates of refractoriness. This can add pressure on platelet supplies, increase workload, and raise overall costs. These issues are especially important for low- and middle-income countries, where resources and infrastructure are often limited. Because of this, the operational impact of PRTs should be interpreted with caution.

Overall, the randomized studies agreed that PRT-treated platelets give lower 1-hour and 24-hour corrected count increments (CCI) compared to untreated platelets. This was shown in many important studies, including Garban et al. (2018), Rebulla et al. (2017), van der Meer et al. (2018), and Brixner et al. (2021).

These studies consistently showed that treated platelets, whether processed with amotosalen-UVA, riboflavin-UV, or UVC light had smaller increases after transfusion. This lower increase is thought to happen because the PRT process puts stress on the platelets and

causes some changes in their structure. However, it is important to note that the amount of decrease was not the same for all PRT systems. Some systems had smaller effects than others. In this work, photochemical PRT systems such as amotosalen-UVA and riboflavin-UV, which rely on additive molecules, are explicitly distinguished from photophysical UVC systems that operate without chemical photosensitizers; this distinction may explain the differing outcomes observed in the Brixner et al. UVC trial. Despite the reduced CCI values, the trials did not discover notable variations in clinical hemostasis, showing that platelet function is conserved sufficiently to maintain acceptable bleeding control.

Clinical bleeding outcomes comprised a significant aspect of the study assessments. Most of the high-quality randomized trials reported no significant increase in WHO grade ≥2 hemorrhage in patients receiving PRT-treated platelets. For example, Garban et al. (2018) observed identical rates of clinically significant bleeding across intervention and control groups in the EFFIPAP study. Likewise, van der Meer et al. (2018) found that in intention-to-treat analysis, pathogen-reduced platelets were non-inferior to untreated platelets in avoiding moderate to severe bleeding. Findings from systematic reviews such as Estcourt et al. (2017) and Pati et al. (2022) further reinforced this finding, revealing that although PRT platelets lower laboratory increases, they do not significantly impair clinical bleeding outcomes. Therefore, it can be claimed that, across various trials and circumstances, PRT maintains hemostatic effectiveness at a clinically acceptable level.

Safety outcomes were also comparable across research. Hemovigilance systems, particularly large observational datasets such as the one described by Knutson et al. (2015), demonstrated relatively low transfusion-reaction rates using PRT platelets. In their research, the rate of acute transfusion responses was just 0.6%, with significant adverse effects (<0.1%) seldom ascribed to transfusion. Similar outcomes were observed in observational and post-implementation studies in diverse locations, including those by Piotrowski et al. (2018), Schulz et al. (2019), and Bahar et al. (2020). These trials observed constant rates of febrile, allergic, and other transfusion responses despite shifting to pathogen-reduced components. In addition, PRTs offer a substantial advantage by essentially preventing many transfusion-transmitted infections, a benefit confirmed both clinically and in laboratory investigations such as Rustanti et al. (2020). Because of this, PRTs continue to be considered as a major safety enhancer, especially in circumstances where the danger of infection transmission is considerable.

Although most data agreed well, considerable variance and outliers were detected. In certain studies, notably per-protocol analysis, non-inferiority margins for bleeding outcomes were not satisfied. For example, the per-protocol arm of the van der Meer et al. (2018) study indicated slightly greater bleeding levels in the PRT group, even though the intention-to-treat analysis fulfilled non-inferiority criteria. Similarly, Rebulla et al. (2017) showed greater platelet utilization and smaller increments utilizing both amotosalen and riboflavin systems. These data demonstrate that while PRT is typically beneficial, some technologies or patient subgroups may exhibit lower effectiveness that must be monitored. Another area of difference involves platelet usage: numerous trials showed that patients receiving PRT platelets required more frequent transfusions or higher numbers of units, with some studies indicating increases of up to 50% (Brixner et al., 2021). This implies that while PRT provides safety, it may raise operational demands and resource consumption in blood banks. The observed increase in platelet utilization following PRT adoption suggests that hospitals transitioning to 100% PRT may need to reinforce inventory management processes, including maintaining adequate buffer stocks to prevent supply strain

Differences across PRT technology also contributed to variances in outcomes. Amotosalen-UVA and riboflavin-based systems have been the most intensively investigated, with fairly consistent effects across varied populations. In contrast, newer systems like UVC-based PRT exhibited somewhat increased rates of refractoriness (repeated inadequate post-transfusion platelet increment despite adequate dosing) and transfusion failure (inability of a platelet unit to achieve the expected rise in platelet count under standard clinical conditions) in certain instances (Brixner et al., 2021). Plasma-specific PRT approaches, such as the methylene blue system examined by Arroyo et al. (2020), revealed clinical efficacy equivalent to conventional plasma, albeit some previous studies reported minor decreases in coagulation factors. These technological variations underscore the need of selecting PRT systems based on the demands and capabilities of the blood service or clinical setting.

The consequences of these discoveries are substantial for blood banks and transfusion programs. The most essential advantage of PRT usage is better blood safety, notably in reducing transfusion-transmitted illnesses from known and developing pathogens. This is particularly useful in nations where screening infrastructure is poor or when donor populations have increased infection risks. However, deployment of PRT may also lead to greater platelet consumption, which might strain inventories and raise

expenses. Blood banks must plan for these logistical problems while acknowledging the long-term safety advantages. Furthermore, certain PRT systems offer prolonged storage, such as 7-day shelf life for platelets, which can enhance supply management and minimize waste (Pedraza et al., 2024). Thus, PRT can both improve and challenge blood systems, depending on how resources are utilized.

For national blood safety programs, PRT provides an extra protection beyond regular testing procedures. It decreases reliance on donor deferral procedures, mitigates risk from developing diseases, and adds consistency to transfusion safety even during outbreaks. Hemovigilance data across multiple locations indicate PRT as a viable strategy for minimizing bacterial contamination occurrences and enhancing patient protection (Knutson et al., 2015; Piotrowski et al., 2018). These benefits may assist drive policy and long-term planning for transfusion services, especially in impoverished nations or high-risk contexts.

Patients also stand to gain from the usage of PRT-treated blood components. Despite decreased laboratory increments, clinical results stay steady, and the chance of obtaining a safer product is substantially higher. Patients with weaker immune systems, such as those undergoing cancer therapy or stem cell transplantation, are particularly safeguarded by PRT's capacity to inactivate viruses, germs, and parasites. Although certain patients may require more frequent transfusions due to lesser increments, the overall risk profile remains extremely favorable, and this trade-off is frequently acceptable in practical practice.

Study Limitations

This systematic review has significant limitations that should be noted. First, the included research differed in design, sample size, and population characteristics. Randomized controlled trials exhibited excellent internal validity, however numerous observational and hemovigilance studies lacked randomization and blinding, which may add bias. Second, discrepancies across PRT technology make direct comparison problematic. Systems such as amotosalen-UVA, riboflavin-UV, and UVC employ distinct pathways, and their efficacy differs between results.

Another problem is that several research focused mostly on laboratory markers rather than clinically significant bleeding events. Similarly, not many trials disclosed precise safety data such as particular transfusion-reaction subtypes or long-term outcomes. There were also geographical disparities, since some locations have more advanced blood screening methods, while others rely largely on PRT due to increased infection dangers. Although the review includes data from the Asian region, most of the evidence is derived from laboratory inactivation studies, and clinical outcome data from Asian patient populations remain limited; thus, clinical generalizability for this region should be interpreted cautiously

Lastly, publication bias may be present because most research come from well-resourced environments, and some unfavorable findings may remain unreported. These criteria should be addressed when assessing the generalizability of the findings.

Conclusion

This systematic analysis investigated the clinical efficacy and safety of pathogen-reduction technologies (PRTs) utilized for platelets and plasma across studies published from 2015 to 2025. The overall findings suggest that although PRT-treated platelets regularly offer reduced laboratory increments—specifically lowered corrected count increment (CCI) values—they nonetheless demonstrate appropriate clinical hemostatic efficacy in most patients. Clinical bleeding outcomes, notably WHO grade ≥2 hemorrhage, were typically comparable between PRT and traditional platelets in the majority of randomized trials and observational studies. Furthermore, PRT-treated plasma also maintained excellent therapeutic responses across diverse clinical applications.

Safety findings were extremely positive across all examined trials. Transfusion responses were modest, and significant adverse outcomes were infrequent. Large hemovigilance datasets supported the good safety profile of PRT components, while laboratory investigations indicated robust inactivation of a wide spectrum of pathogens. This makes PRT an important technique for boosting transfusion safety, especially in locations with high infectious disease burden or limited screening capabilities. However, problems were also discovered. Several studies found greater platelet consumption and, in certain situations, higher transfusion failure rates related with specific PRT methods. These outcomes can boost operational expenses and impose additional strain on blood bank inventory. Technology-specific variances also exist, as certain newer systems (e.g., UVC-based PRT) demonstrated somewhat worse performance in selected outcomes. Given the heterogeneity in PRT technologies, study designs, and outcome reporting, the conclusions of this review should be interpreted cautiously, and future comparative trials are needed to better define technology-specific performance

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Authors' Contributions:
Oluchi Okechukwu conceived the study, coordinated the systematic review process, and drafted the main sections of the manuscript. Oluchi Okechukwu and Chidinma Gab-Obinna contributed to the study design and literature screening. Adepeju Kafayat Olowookere and Christopher Bijabdo Jato performed data extraction and quality appraisal of included studies. Richard Afriyie Osei and Dorcas Okayo Okoroafor contributed to data synthesis and interpretation of findings. Lawrence John Ajutor and Tobi David Farinde assisted with database searching and reference management. All authors critically reviewed, edited, and approved the final manuscript.
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Review Article

Next-Generation Sequencing for Minimal Residual Disease Detection in AML: Current Technologies and Clinical Implications

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Abstract:
Minimal residual disease (MRD) has become a significant predictor of relapse and survival in acute myeloid leukemia (AML), indicating the extent of remission beyond traditional morphological evaluation. Although multicolor flow cytometry and quantitative PCR are essential methodologies in minimal residual disease identification, both are constrained by immunophenotypic variability, the necessity for stable molecular targets, and limited sensitivity. Advancements in next-generation sequencing (NGS) have revolutionized the minimal residual disease (MRD) field by enabling highly sensitive, mutation-driven identification of leukemic clones across a broad genomic landscape. Contemporary error-suppressed next-generation sequencing techniques—such as unique molecular identifiers, duplex sequencing, and single-molecule molecular inversion probes—have enhanced analytical sensitivity to the 10^{-5} to 10^{-6} range, enabling the detection of ultra-low-frequency variations with greater specificity. These techniques improve clinical risk classification, refine prognostication within genetically defined AML subtypes, and guide therapeutic options, including post-remission therapy, targeted inhibition, and the timing and intensity of allogeneic stem cell transplantation. Innovative applications, such as single-cell sequencing, cell-free DNA studies, and integrative multi-omic MRD evaluation, enhance the capabilities of genomics-based monitoring. Nonetheless, obstacles remain, such as differentiating cancer mutations from clonal hematopoiesis, standardizing analytical pipelines, establishing clinically relevant thresholds, and incorporating NGS MRD into standardized treatment protocols. This review encapsulates contemporary NGS methods for AML MRD diagnosis, assesses their clinical ramifications and constraints, and suggests future pathways necessary for comprehensive clinical integration. With advancements in the area, NGS-based MRD is set to become a pivotal element of precision-guided AML control.

Keywords: Minimal Residual Disease (MRD); Acute Myeloid Leukemia (AML); Next-Generation Sequencing (NGS); Error-Suppressed Sequencing; Clonal Hematopoiesis

Introduction

Acute myeloid leukemia (AML) is a biologically and clinically heterogeneous hematologic malignancy characterized by the clonal expansion of aberrant myeloid progenitors. Its genomic landscape includes recurrent driver mutations, epigenetic alterations, and chromosomal abnormalities that shape disease phenotype, therapeutic response, and prognosis. Large-scale sequencing efforts have shown that AML evolves through a hierarchical architecture of founder and subclonal mutations, contributing to substantial inter- and intra-patient variability. This complexity underscores the need for refined biomarkers that can guide risk-adapted treatment strategies beyond conventional morphologic assessment [1,2].

Minimal residual disease (MRD)—the persistence of leukemic cells below the threshold of microscopic detection—has emerged as one of the strongest predictors of relapse and survival in AML. MRD positivity after induction or consolidation therapy correlates with significantly higher recurrence rates, inferior overall survival, and adverse post-transplant outcomes. Consequently, MRD assessment is increasingly incorporated into clinical trials, risk stratification systems, and transplant decision-making frameworks. Despite its importance, the optimal timing, methodology, and clinical interpretation of MRD detection remain subjects of active investigation [3,4].

Historically, MRD monitoring has relied on multiparameter flow cytometry (MFC) and quantitative PCR (qPCR). While both methods remain valuable, they have inherent limitations: MFC is affected by immunophenotypic variability and operator-dependent interpretation, and qPCR is restricted to a narrow set of stable, mutation-specific targets such as NPM1 or core-binding factor fusions. These constraints limit their ability to capture the full mutational diversity and clonal complexity that characterize modern AML biology [5].

The advent of next-generation sequencing (NGS) has transformed MRD assessment by enabling sensitive, mutation-informed detection across a broad range of genetic lesions. Error-corrected NGS technologies—including unique molecular identifiers, duplex sequencing, and single-molecule inversion probe methods—can identify ultra-low–frequency variants with high specificity, helping overcome the challenges associated with conventional techniques. As a result, NGS-based MRD has the potential to improve prognostication, refine post-remission risk assessment, and support more individualized therapeutic decision-making [6].

In this review, we summarize the molecular basis of MRD in AML, outline contemporary NGS platforms and analytical approaches, evaluate their clinical applications and limitations, and discuss emerging technologies poised to shape next-generation MRD monitoring. Together, these advances highlight the expanding role of NGS MRD as a cornerstone of precision AML management [7].

Molecular Basis of MRD in AML

Genetic Landscape of AML Relevant to MRD

The molecular heterogeneity of acute myeloid leukemia (AML) significantly affects disease persistence and relapse patterns, rendering genomic context crucial for interpreting minimal residual disease (MRD) [8]. Acute Myeloid Leukemia (AML) is propelled by recurrent somatic mutations that can be classified into several functional categories, including transcription factor fusions (e.g., RUNX1-RUNX1T1, CBFB-MYH11), mutations in signaling pathways (FLT3-ITD/TKD, KIT, RAS), alterations in epigenetic regulators (DNMT3A, TET2, IDH1/2, ASXL1), mutations in NPM1 exon-12, and genes that influence hematopoietic differentiation (CEBPA, RUNX1)[9]. Certain mutations among them serve as very informative MRD markers due to their stability and specificity to leukemia. NPM1 mutations occur in around one-third of AML cases and generally signify early clonal occurrences, rendering them suitable molecular anchors for MRD surveillance [10].

The mutational architecture of AML generally consists of two primary categories of lesions: initiating (founder) mutations and collaborating (progression) mutations. Initiating lesions, frequently associated with epigenetic modifiers like DNMT3A or TET2, create preleukemic clones that may endure despite the effective elimination of leukemic blasts [11]. Conversely, collaborating lesions like NPM1, FLT3-ITD, or IDH1/2 frequently propel leukemogenesis and delineate the predominant leukemic clone at the time of diagnosis. Due to their heightened specificity for leukemia and stronger correlation with disease activity, these cooperative mutations frequently represent the most clinically significant targets for MRD detection. Comprehending this hierarchy is crucial for understanding residual variations and distinguishing between persistent malignant clones and background clonal hematopoiesis [12].

Clonal Evolution and Implications for MRD Detection

The course and relapse of AML are influenced by dynamic clonal evolution, as selective forces from chemotherapy, targeted therapy, and the immunological microenvironment alter the composition of leukemic subclones. Founder mutations, usually associated with genes that govern epigenetic regulation or self-renewal, frequently endure despite treatment owing to their functional significance in initial clonal growth and their existence in preleukemic hematopoietic stem cells. The detection of these lesions in long-lived nonmalignant hematopoietic cells indicates that their persistence does not inherently denote active leukemia, presenting a significant challenge for MRD interpretation [13]. Acute myeloid leukemia (AML) is marked by dynamic clonal evolution in response to treatment pressure, where founder and subclonal mutations affect the probability of relapse (Figure 1).

Conversely, subclonal mutations that arise during leukemic transformation or illness development may serve as more sensitive indications of residual disease. This includes mutations obtained during clonal diversification—such as FLT3-ITD, RAS-pathway mutations, or other cooperative events—that may be eliminated with effective treatment or may re-emerge under therapeutic pressure. Relapse often arises from tiny subclonal populations that are present at diagnosis but undetectable by standard testing. High-sensitivity NGS technologies facilitate the identification of low-frequency subclones, enhancing relapse prediction and guiding therapy approaches[14].

A significant obstacle in NGS-based MRD evaluation is differentiating malignant clones from age-associated clonal hematopoiesis (CHIP). CHIP-related mutations, predominantly in DNMT3A, TET2, and ASXL1, may endure or even proliferate with chemotherapy; however frequently do not indicate actual leukemic burden. Their existence affects the interpretation of MRD data, especially when identified at low variant allele frequencies in remission samples. Advanced analytical methodologies, encompassing the integration of variant allele frequency dynamics, identification of co-occurring leukemic-specific mutations, and incorporation of immunophenotypic or clinical context, are crucial for distinguishing CHIP from MRD and preventing overtreatment [15].

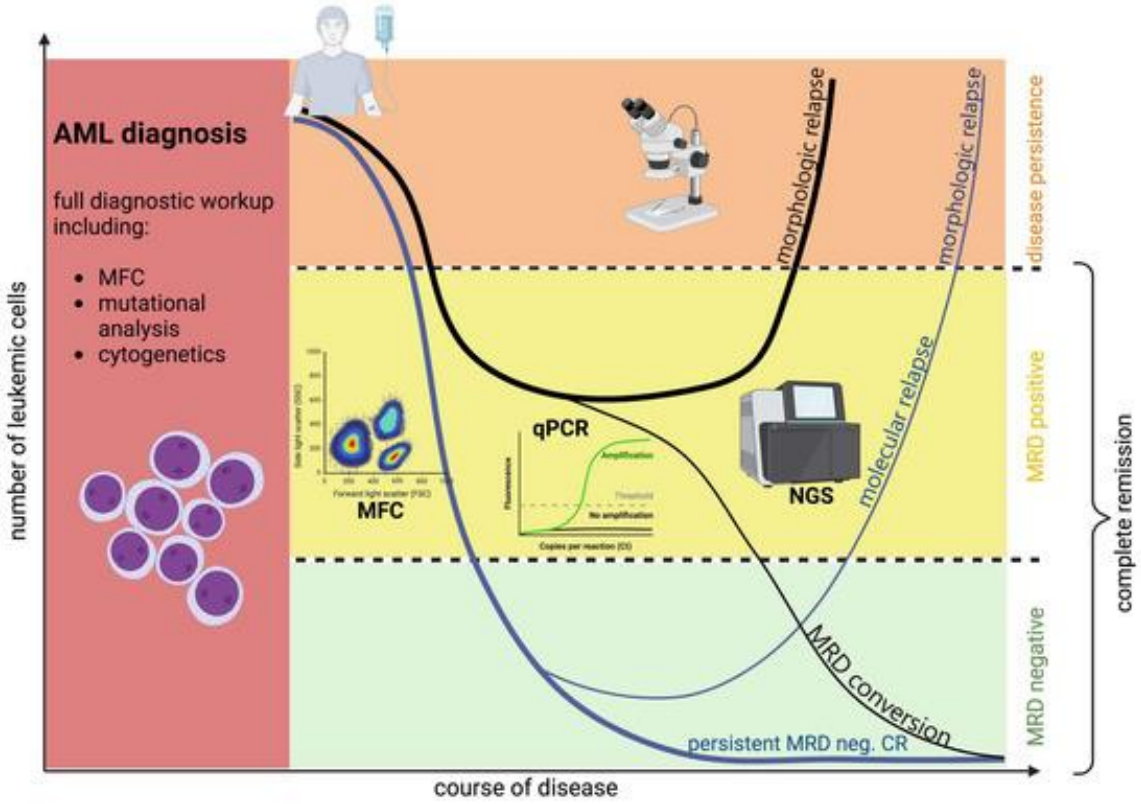

Figure 1. Overview of MRD detection in AML. (A) Clonal architecture showing founder and subclonal mutations and therapy-induced selection. (B) Sensitivity of MRD detection methods: MFC, qPCR/dPCR, targeted NGS, and error-corrected NGS. (C) Clinical integration: post-induction/consolidation, pre-/post-HSCT, targeted therapy monitoring, and relapse prediction. Adapted from Moritz J. et al., Biomedicines 2024;12(3):599.

Next-Generation Sequencing Platforms and Approaches for MRD Detection

Next-generation sequencing (NGS) has revolutionized MRD detection in acute myeloid leukemia (AML) by enabling sensitive, mutation-informed monitoring beyond the capabilities of conventional assays. Multiple NGS platforms and methodological approaches have been developed, each with distinct advantages, limitations, and clinical applications.

Amplicon-Based Targeted NGS

Amplicon-based NGS utilizes PCR amplification of specific genomic areas to identify mutations with high depth of coverage. Its principal advantages are rapid turnaround, substantial sequencing depth, and economic efficiency, facilitating the sensitive identification of low-frequency variations, generally ranging from 0.1% to 1% variant allele frequency (VAF). Amplicon methodologies are extensively employed for the identification of prevalent recurrent mutations, including NPM1, FLT3, and IDH1/2. Limitations encompass constrained genome coverage, vulnerability to PCR amplification bias, and diminished accuracy in GC-rich or intricate areas. Notwithstanding these limitations, amplicon-based NGS is exceptionally appropriate for clinical MRD surveillance in AML cases with well-defined mutations [16].

Hybrid Capture–Based Targeted NGS Panels

Hybrid capture panels employ probes to enhance genomic regions of interest before sequencing, thereby offering extensive coverage of mutation hotspots and structural variants. In contrast to amplicon-based techniques, hybrid capture allows for the concurrent analysis of several genes, hence enhancing the identification of coexisting subclonal mutations and structural variations. Performance characteristics generally attain sensitivities between 0.1% and 0.5% VAF in the absence of specialist error suppression. Although hybrid capture is slower and more expensive than amplicon methods, it provides greater flexibility and is especially advantageous for intricate AML genomes or research-focused MRD evaluation [17].

Error-Suppressed Sequencing Technologies

- Error-suppressed NGS techniques have developed to address technical constraints and attain ultra-sensitive MRD detection, achieving limits of 10^{-5}–10^{-6}. Essential tactics encompass:
- Unique Molecular Identifiers (UMIs): Labeling individual DNA molecules facilitates the distinction between genuine variations and sequencing mistakes.
- Duplex Sequencing (DS): Both strands of DNA are sequenced, facilitating error correction and nearly full eradication of false positives.
- Single-Molecule Molecular Inversion Probes (smMIPs) provide precise and efficient targeted sequencing of certain genomic regions.

These techniques are especially beneficial for tracking low-burden subclones and post-transplant minimal residual disease (MRD), when conventional next-generation sequencing (NGS) fails to provide adequate sensitivity [18,19].

Whole-Exome and Whole-Genome Sequencing

Whole-exome (WES) and whole-genome sequencing (WGS) provide comprehensive mutation detection across the coding genome or entire genome, respectively. While invaluable for research into clonal evolution and the discovery of novel driver mutations, their clinical utility for MRD is limited. The primary constraints are lower depth of coverage, high cost, extended turnaround times, and insufficient sensitivity for low-frequency variants (<1%). Consequently, WES/WGS are currently complementary to targeted NGS in MRD studies rather than routine clinical tools [20].

RNA-Based Sequencing Methods

RNA sequencing facilitates the identification of fusion transcripts, alternative splicing events, and variations in expression levels linked to leukemic clones. It is especially pertinent for the surveillance of NPM1-mutated acute myeloid leukemia, KMT2A fusions, and other transcript-based anomalies. RNA-based methodologies provide the benefit of identifying disease-specific transcripts in the absence of genomic alterations; nevertheless, RNA degradation and fluctuating expression levels may constrain sensitivity and repeatability [21].

3.6 Bioinformatic Pipelines for Ultra-Low–Frequency Variant Detection

Precise MRD evaluation depends on advanced computational techniques to differentiate genuine variants from sequencing artifacts and clonal hematopoiesis. Essential factors to consider are:

- Error Modeling: Quantitative frameworks to address sequencing and PCR inaccuracies.
- Variant Filtering: Approaches to eliminate technological artifacts while preserving authentic low-frequency mutations.
- CHIP Discrimination: Incorporation of longitudinal VAF trends and co-mutation

patterns to differentiate pre-leukemic clones from malignant minimal residual disease (MRD).

- Standardization Challenges: Variations in pipelines, variant calling thresholds, and reporting formats hinder cross-study comparability, underscoring the necessity for unified bioinformatic standards.

This collection of NGS platforms and methodologies offers a range of choices for MRD detection, encompassing targeted, clinically relevant tests and extensive research-oriented analysis. The selection of a platform is contingent upon the type of mutation, required sensitivity, sample type, and clinical context, underscoring the necessity of incorporating both technical and biological factors in NGS-based AML MRD monitoring [18,22].

Several NGS platforms are available for MRD monitoring, each with distinct sensitivity and coverage characteristics (Table 1)."

Taken together, these NGS approaches form a graduated spectrum of depth, breadth, and sensitivity. Amplicon sequencing and hybrid-capture panels are the most clinically mature and widely implemented modalities, whereas UMI- and duplex-based error-suppressed methods provide the greatest analytical sensitivity but currently face practical challenges related to cost, turnaround time, and workflow complexity. In contrast, whole-exome and whole-genome sequencing remain primarily research tools due to insufficient depth for low-level MRD. Recognizing how these approaches complement one another helps contextualize their role in clinical MRD monitoring.

Table 1. Comparison of MRD Detection Modalities in AML

Modality	Sensitivity	Coverage	Turnaround	Strengths	Limitations	Typical Clinical Use
NGS (amplicon-based)	0.1–1% VAF	Selected recurrent genes	Fast	Cost-effective, high depth	Limited gene panel, PCR bias	Routine MRD for NPM1, FLT3, IDH1/2
NGS (hybrid capture)	0.1–0.5% VAF	Broad panel (50–500 genes)	Moderate	Captures subclones & structural variants	Higher cost, longer prep	Complex AML genomes, research
Error-suppressed NGS (UMI/DS/smMIP)	10^{-5}–10^{-6}	Targeted	Slow	Ultra-sensitive, tracks rare subclones	Expensive, complex workflow	Post-transplant MRD, early relapse prediction
Flow Cytometry (MFC)	0.01–0.1%	Immunophenotype	Fast	Widely available, real-time	Immunophenotypic drift, operator-dependent	Routine clinical MRD
qPCR / Digital PCR	10^{-5}–10^{-6}	Specific mutations/fusions	Fast	High sensitivity, quantitative	Limited to defined targets	NPM1, RUNX1-RUNX1T1, CBFB-MYH11

Analytical Performance and Standardization Challenges

Despite the transformative potential of next-generation sequencing (NGS) for MRD detection in AML, several analytical and standardization considerations must be addressed to ensure clinical reliability and reproducibility. These factors determine assay sensitivity, interpretability, and comparability across laboratories, which are essential for integrating NGS MRD into routine clinical practice.

Sensitivity, Specificity, and Reproducibility

NGS-based MRD detection exhibits exceptional analytical sensitivity, especially when integrated with error-suppression techniques, frequently exceeding 0.01% variant allele frequency (VAF) in optimized

procedures. In contrast to multiparameter flow cytometry (MFC), which generally identifies leukemic cells at 0.1–0.01%, next-generation sequencing (NGS) offers extensive coverage of mutational targets and can find subclonal variants overlooked by immunophenotypic methods. Quantitative PCR (qPCR) is the benchmark for detecting highly recurring mutations like NPM1, with sensitivities reaching as low as 10^{-5}–10^{-6}; yet, qPCR is restricted to particular targets. NGS integrates the extensive capabilities of MFC with near-qPCR sensitivity for specific mutations, providing consistent detection across longitudinal samples. Inter-assay repeatability may fluctuate based on library preparation, sequencing depth, error-correction techniques, and bioinformatic workflows [19].

Further strengthening of NGS MRD will require coordinated multi-institutional validation efforts to ensure reproducibility across platforms and laboratories. The development of standardized reference materials and clinically calibrated control samples is especially important for evaluating assay sensitivity and error-suppression performance. Large, prospective studies comparing NGS MRD against established clinical outcomes will be essential to define the analytical robustness required for routine implementation.

Limit of Detection and Clinical Threshold Settings

The clinical interpretation of NGS MRD necessitates the establishment of thresholds that forecast relapse risk. Numerous studies demonstrate that persistent mutations exceeding 0.1–1% VAF following induction or consolidation correlate with markedly elevated recurrence rates, while the thresholds are individual to each mutation and affected by the illness setting. Ultra-sensitive detection at levels of 10^{-5}–10^{-6} can identify developing subclones months prior to clinical relapse, providing a potential opportunity for preventative intervention. Nonetheless, establishing clinically actionable thresholds is difficult due to the persistence of pre-leukemic clones or clonal hematopoiesis, which may not lead to manifest illness [23].

Across contemporary clinical trials, MRD thresholds vary by mutation type and treatment phase, but several patterns have become consistently recognized. For NPM1-mutated AML, many ELN- and trial-based protocols define a threshold of approximately 10^{-3} (0.1% NPM1 copies/ABL1 or <1% VAF) after induction as clinically significant, with deeper thresholds of 10^{-4}–10^{-5} associated with improved relapse-free survival following consolidation [10,26]. In contrast, FLT3-ITD is typically monitored using a VAF-based cutoff of *0.01–0.05%* in post-remission settings, with persistent or rising FLT3-mutant clones above these ranges correlating with early relapse and poorer transplant outcomes [14,23]. For fusion transcripts such as RUNX1-RUNX1T1 and CBFB-MYH11, qPCR-anchored trial frameworks often use ≥1-log increase from prior nadir or transcript levels >10^{-3} as high-risk markers [3,26]. Consolidating these widely referenced cutoffs highlights the ongoing need for standardized thresholds but also provides practical guidance for interpreting MRD dynamics across commonly monitored AML genotypes.

Pre-Analytical Variables

The type of sample and the timing of collection substantially affect MRD detection. Bone marrow aspirates offer superior sensitivity due to the concentration of leukemic cells compared to peripheral blood; however, serial blood-based monitoring is less intrusive and may detect circulating subclones. The ideal timing for MRD evaluation differs according to treatment phase: post-induction, post-consolidation, and pre-transplant assessments are pivotal points for prognostication and therapy decisions. Inconsistent or delayed sampling may result in the underestimation of residual disease and jeopardize clinical interpretation [24].

Inter-Laboratory Variability and Need for Harmonization

Standardization among laboratories is crucial for the integration of NGS MRD into clinical practice. Variability stems from discrepancies in sequencing platforms, library preparation techniques, bioinformatic workflows, and reporting standards. The implementation of reporting systems, including defined variant allele frequency thresholds and minimal residual disease positive criteria, is essential for comparison. The utilization of reference standards, such as synthetic DNA controls and thoroughly defined clinical samples, enhances test validation and external quality assessment. Collaborative efforts among multiple institutions are in progress to develop standardized protocols, enhance quality assurance, and minimize discrepancies in MRD reporting, which will be essential for regulatory endorsement and clinical implementation [25].

Synopsis: The analytical performance and standardization are pivotal to the clinical applicability of NGS MRD. Although high sensitivity, specificity, and repeatability establish NGS as an exceptional method for AML MRD diagnosis, meticulous attention to clinical thresholds, sample management, and standardized processes is essential to guarantee precise, interpretable, and similar outcomes across institutions.

Overall, much of the perceived complexity surrounding NGS MRD reflects practical barriers such

as laboratory workflow variability, cost, and turnaround time rather than theoretical limitations of the sequencing technologies themselves. Continued harmonization efforts, supported by standardized bioinformatic pipelines and reference materials, will be essential to ensure that clinically mature NGS approaches can be deployed consistently across institutions."

Clinical Utility of NGS-Based MRD in AML

NGS-based minimal residual disease (MRD) assessment has rapidly emerged as a transformative tool in acute myeloid leukemia (AML), offering prognostic precision and guiding therapeutic decision-making. Its clinical utility spans risk stratification, post-remission management, monitoring of targeted therapy response, and early relapse prediction.

Prognostic Value Across AML Subtypes

The prognostic significance of NGS MRD differs among genetically categorized AML subsets:

- NPM1-mutated acute myeloid leukemia (AML): The retention of NPM1-mutated clones during induction or consolidation significantly forecasts relapse and decreased survival outcomes. Next-generation sequencing facilitates the precise identification of remaining NPM1 mutations, even at ultra-low frequencies ($<10^{-4}$), enhancing post-remission risk assessment [7].
- Core-binding factor acute myeloid leukemia (CBF-AML): Minimal residual disease (MRD) detection with next-generation sequencing (NGS) targeting RUNX1-RUNX1T1 or CBFB-MYH11 fusions is associated with recurrence risk, enhancing quantitative polymerase chain reaction (qPCR)-based tests and offering insights into subclonal evolution.
- FLT3-mutated AML: Due to the significant recurrence tendency of FLT3-ITD–positive AML, NGS MRD can identify persistent or developing FLT3 subclones that may require preemptive intervention or intensification of therapy.
- Secondary and therapy-related AML: Monitoring minimal residual disease (MRD) in these genetically intricate subtypes poses challenges; however, next-generation sequencing (NGS) for detecting cooperating mutations or enduring founder clones has shown prognostic significance, identifying patients at heightened risk of relapse despite morphologic remission [26].

Common driver and cooperating mutations vary in their suitability for MRD monitoring (Table 2)."

Table 2. Mutation-Specific MRD Relevance in AML

Mutation / Alteration	MRD Suitability	Prognostic Value	Recommended MRD Method	Notes
NPM1	High	Strong predictor of relapse	NGS, qPCR	Stable founder mutation; widely used in trials
FLT3-ITD / FLT3-TKD	Moderate	High relapse risk	NGS, dPCR	Subclonal dynamics are important; they may guide targeted therapy
DNMT3A	Low	Often, CHIP has limited predictive value	NGS	Pre-leukemic clone; persistent post-therapy
RUNX1-RUNX1T1	High	Predicts relapse in CBF-AML	qPCR, RNA-seq, NGS	Fusion transcript allows sensitive monitoring
CBFB-MYH11	High	Prognostic in CBF-AML	qPCR, RNA-seq, NGS	Strong MRD marker post-consolidation
IDH1/2	Moderate	Guides targeted therapy	NGS	Useful for therapy monitoring; may emerge as resistance
TET2 / ASXL1	Low	CHIP; limited MRD utility	NGS	Not recommended as sole MRD marker

Guiding Treatment Decisions

NGS MRD informs multiple therapeutic decisions:

- Risk stratification following induction and consolidation: MRD-positive status indicates patients at high risk of relapse despite achieving morphologic remission,

informing intensification methods or the consideration of early allogeneic hematopoietic stem cell transplantation (HSCT).

- **Optimization of HSCT:**
- Pre-transplant minimal residual disease (MRD) forecasts relapse and post-transplant survival. Patients exhibiting detectable minimal residual disease frequently derive advantages from intensified conditioning or supplementary bridging therapy.
- Post-transplant minimal residual disease (MRD) facilitates early management by donor lymphocyte infusions (DLI), immunomodulation, or targeted treatments, potentially averting overt recurrence and enhancing long-term outcomes [19,27].

An additional scenario in which NGS MRD is increasingly relevant is the management of older or unfit patients treated with hypomethylating agents combined with venetoclax. Early studies suggest that persistent MRD—particularly NPM1 or FLT3-mutant clones—after several treatment cycles correlates with early relapse, while deep remissions may help identify patients who can transition to lower-intensity maintenance strategies. As HMA/ven regimens become standard of care in this population, NGS MRD may aid in treatment tailoring and in determining duration of therapy.

Monitoring Response to Targeted Therapies

NGS MRD facilitates dynamic monitoring of disease burden during targeted therapy:

- **FLT3 inhibitors:** Detection of emerging FLT3-mutant subclones can indicate resistance or incomplete response, informing dose adjustment or combination strategies.
- **IDH1/2 inhibitors:** MRD tracking can guide treatment continuation or escalation, particularly in patients with low-burden residual disease.
- **Menin inhibitors:** For MLL-rearranged or NPM1-mutated AML, NGS MRD allows early identification of therapeutic escape and supports adaptive treatment strategies.

Early Relapse Detection and Prediction

NGS MRD offers a significant lead-time advantage over conventional flow cytometry by detecting ultra-low–frequency leukemic clones months before clinical relapse. Integration of NGS MRD into longitudinal surveillance enables:

- Timely preemptive interventions to prevent hematologic relapse.
- Identification of clonal evolution and emergent resistance mutations.
- Personalized monitoring schedules based on mutational profile, treatment intensity, and transplant status [28].

Summary: NGS MRD provides robust prognostic information, informs treatment decisions across AML subtypes, guides post-transplant management, and enables early detection of relapse. Its integration into routine clinical practice promises a precision-guided approach to AML therapy, improving risk-adapted outcomes and optimizing the use of targeted interventions.

Comparisons with Other MRD Modalities

Multiple modalities are employed for minimal residual disease (MRD) detection in AML, each with distinct advantages, limitations, and clinical applicability. Understanding these differences is essential for selecting the optimal method or integrating complementary approaches.

Multicolor Flow Cytometry (MFC)

MFC identifies anomalous immunophenotypic patterns in leukemic blasts, facilitating MRD evaluation in most AML patients. Its advantages encompass swift execution, extensive applicability, and the capacity to identify phenotypically characterized leukemic populations irrespective of particular mutations. MFC generally attains sensitivities of 0.01–0.1%, comparable to conventional NGS for high-burden diseases. Nonetheless, it possesses significant limitations compared to NGS: immunophenotypic plasticity and therapy-induced antigen modulation can obscure leukemic cells, inter-laboratory reproducibility is problematic, and it exhibits reduced sensitivity for identifying rare subclones or differentiating pre-leukemic from malignant populations [29].

Quantitative PCR (qPCR) and Digital PCR (dPCR)

qPCR and dPCR have exceptional sensitivity for minimal residual disease (MRD) identification, achieving limits of 10^{-5} to 10^{-6} for recurrent, well-defined targets, including NPM1 mutations and fusion transcripts (RUNX1-RUNX1T1, CBFB-MYH11). These methodologies provide accurate quantification and defined prognostic thresholds, although they are limited to patients possessing particular genetic mutations. Furthermore, qPCR necessitates standard curves and meticulous calibration, whereas dPCR provides absolute quantification with enhanced repeatability and less technical variability. In

comparison to NGS, these PCR-based techniques exhibit great sensitivity but are limited in their application for heterogeneous or subclonal AML [3,30].

Integrated MRD Assessment

The integration of NGS with MFC or PCR harnesses the synergistic advantages of each technique, facilitating a more thorough assessment of MRD. Integrated methodologies can identify residual disease overlooked by a singular technique, resolve inconsistencies between molecular and immunophenotypic minimal residual disease, and offer comprehensive risk classification. Algorithms integrating genetic and immunophenotypic data are progressively utilized in clinical trials to inform therapy intensification, transplantation decisions, and post-remission surveillance. This multi-modal MRD assessment improves sensitivity, specificity, and prognosis accuracy, establishing a basis for tailored, MRD-focused AML therapy.

Synopsis: Although MFC and PCR-based techniques are important for MRD detection, NGS provides extensive genome coverage, enhanced sensitivity for subclonal populations, and the capability to track dynamic clonal evolution. The integration of various MRD modalities offers a synergistic strategy, enhancing detection accuracy and guiding precision-targeted treatment actions [31].

Emerging Technologies and Evolving Concepts

Advances in molecular profiling and computational biology are expanding the landscape of minimal residual disease (MRD) monitoring in AML. Novel technologies promise to enhance sensitivity, capture clonal complexity, and enable predictive modeling that may transform personalized disease management.

Single-Cell NGS for Clonal Mapping

Single-cell sequencing facilitates high-resolution characterization of individual leukemic cells, permitting the discovery of rare subclones that may resist treatment. Single-cell NGS elucidates the hierarchical and temporal evolution of AML clones by reconstructing clonal architecture, thereby identifying which subclones are predisposed to induce relapse. This method enables accurate MRD tracking, guiding focused intervention tactics and providing the capability to observe therapy-induced clonal selection with exceptional resolution [32].

Cell-Free DNA for MRD Monitoring

Circulating tumor DNA (ctDNA) in peripheral blood represents a non-invasive biomarker for MRD. Early studies demonstrate that ctDNA can detect residual leukemic clones, including those undetectable by bone marrow sampling, and may provide a lead-time advantage for relapse prediction. While challenges remain—including assay sensitivity, standardization, and distinguishing malignant DNA from clonal hematopoiesis—cell-free DNA offers a promising avenue for serial, minimally invasive MRD surveillance, particularly in patients unsuitable for frequent bone marrow assessments [33].

Multi-Omic MRD Strategies

Integrative multi-omic approaches combine genomic, epigenomic, transcriptomic, and proteomic data to enhance MRD detection and biological interpretation. Epigenetic MRD assessment, such as methylation profiling, can detect residual leukemic signatures that persist even in the absence of detectable mutations. Proteogenomic strategies leverage aberrant protein expression alongside mutational data, providing functional context to MRD and potentially identifying therapy-responsive or resistant subclones. Such multi-dimensional analyses have the potential to capture residual disease comprehensively and improve relapse risk stratification [34].

Although these emerging approaches offer unprecedented resolution and biological insight, each will require rigorous clinical-grade validation before adoption. Benchmarking single-cell sequencing, ctDNA-based MRD, and multi-omic assays against existing NGS and PCR modalities will be crucial to determine their sensitivity, reproducibility, and real-world clinical value. Current barriers, including cost, limited input material for ctDNA, and computational complexity, must be addressed for these technologies to advance toward standardized MRD testing.

AI and Machine Learning in MRD Prediction

Artificial intelligence (AI) and machine learning (ML) are progressively utilized in MRD data to improve predicted precision and assimilate intricate longitudinal information. Algorithms can simulate variation dynamics, identify early indicators of recurrence, and categorize patients according to individualized risk profiles. AI-driven analysis of serial NGS data may differentiate benign clonal hematopoiesis from clinically significant minimal residual disease and enhance the timing of treatment therapies. These computational tools are prepared to enhance precision-guided AML management and inform adaptive treatment regimens [35].

Synopsis: Emerging technologies, such as single-cell NGS, cell-free DNA assays, multi-omic integration, and AI-driven analytics, present unparalleled prospects to enhance MRD diagnosis, comprehend clonal dynamics, and predict relapse. As

these methodologies advance, they are expected to enhance and, in certain scenarios, exceed traditional MRD monitoring, propelling the subsequent evolution of precision AML treatment.

Regulatory, Economic, and Implementation Considerations

The clinical integration of next-generation sequencing (NGS) for minimal residual disease (MRD) monitoring in AML requires consideration of regulatory frameworks, cost-effectiveness, and operational feasibility. These factors are critical to ensure that NGS MRD assays can be reliably adopted in routine clinical practice and produce actionable results for patient management.

Clinical Adoption and Regulatory Landscape

Notwithstanding the increasing evidence endorsing NGS MRD as a predictive and therapeutic instrument, regulatory approvals are still constrained. Current clinical guidelines from organizations like the European LeukemiaNet (ELN) and the National Comprehensive Cancer Network (NCCN) recognize minimal residual disease (MRD) as a vital biomarker; however, they have yet to offer standardized recommendations for next-generation sequencing (NGS)-based MRD assessment. Most commercial and laboratory-developed tests function under CLIA/CAP accreditation, with continuous initiatives to standardize reporting, establish clinically relevant thresholds, and provide reference materials. Regulatory deficiencies encompass the standardization of test sensitivity, repeatability criteria, and explicit directives for incorporating NGS MRD outcomes into therapeutic protocols [22].

Cost-Effectiveness and Health Economics

The adoption of NGS MRD incurs more initial expenses compared to traditional flow cytometry or PCR-based techniques, attributed to sequencing, bioinformatic analysis, and staffing needs. Nevertheless, numerous studies indicate that the capacity of NGS MRD to stratify patients, avert recurrence, and direct tailored therapy may counterbalance these expenses by decreasing hospitalizations, optimizing transplant timing, and circumventing inefficient medications. Cost-effectiveness assessments are increasing, especially in high-risk AML or pre-transplant contexts involving NGS MRD; nonetheless, thorough health economic evaluations are scarce and crucial for widespread implementation [36].

Laboratory Workflow Integration

Operational factors are crucial for the effective clinical deployment of NGS MRD. The turnaround time must correspond with therapeutic decision milestones, specifically during induction, after consolidation, and before transplantation. Efficient sample processing, library preparation, and sequencing workflows are essential, combined with a robust bioinformatic infrastructure capable of detecting ultra-low-frequency variants. Staff must be proficient in molecular methodologies, quality assurance, and data analysis. The standardization of reporting, encompassing variant allele frequencies, MRD positive thresholds, and longitudinal comparisons, is essential for maintaining consistency among institutions. Incorporating MRD results into clinical processes necessitates collaboration with treating physicians to guarantee that these results facilitate prompt therapeutic decisions [37].

Summary: Although NGS MRD possesses transformative potential for AML control, regulatory clarity, cost-effectiveness, and optimized laboratory procedures are essential for its wider implementation. Addressing these problems would guarantee the reliable deployment of NGS MRD to inform tailored treatment regimens and enhance patient outcomes.

Future Directions

Next-generation sequencing (NGS)–based minimal residual disease (MRD) monitoring has demonstrated transformative potential in acute myeloid leukemia (AML), yet several critical areas require further development to realize its full clinical impact.

Harmonized Guidelines and Standardization

The lack of standardized methodologies and reporting frameworks remains a major barrier to clinical adoption. Future efforts must focus on developing harmonized guidelines that define assay performance, limit of detection, and criteria for MRD positivity. Consensus standards will facilitate inter-laboratory comparability, enhance regulatory oversight, and allow reliable integration of NGS MRD into treatment algorithms.

Standardized MRD Thresholds for Intervention

Clinically actionable MRD thresholds are essential to guide therapeutic decisions, including intensification, transplant timing, or preemptive targeted therapy. Large-scale studies are needed to establish mutation-specific and treatment-phase–specific thresholds that reliably predict relapse while minimizing unnecessary interventions [38].

Prospective Clinical Trials Incorporating NGS MRD

Prospective, multi-center trials that integrate NGS MRD as a stratification and response biomarker are critical. Such studies will evaluate the predictive power of NGS MRD for relapse, validate its utility in guiding therapy, and determine the impact of MRD-directed interventions on overall survival and quality of life.

Personalized MRD-Driven Treatment Algorithms

Future clinical practice will increasingly rely on MRD-driven precision strategies. Integration of NGS MRD with genomic, immunophenotypic, and clinical data will enable adaptive treatment algorithms tailored to individual patient risk and clonal dynamics. This approach holds the potential to optimize therapy, minimize toxicity, and improve long-term outcomes in AML.

Summary: The next frontier in AML management involves translating NGS MRD from a prognostic biomarker into a cornerstone of personalized therapy. Achieving this goal requires standardized guidelines, validated thresholds, prospective clinical evidence, and incorporation of MRD into precision-driven treatment strategies.

Conclusions

Next-generation sequencing (NGS) has become an effective instrument for identifying minimal residual disease (MRD) in acute myeloid leukemia (AML), providing exceptional sensitivity, specificity, and the capacity to detect subclonal and changing leukemic populations. Increasing data indicate that NGS MRD offers substantial prognostic insights across many AML subtypes, informs risk-adjusted treatment choices, directs transplant approaches, and facilitates early identification of potential relapse.

In contrast to conventional methods like flow cytometry and PCR, NGS provides extensive genome coverage, improved identification of rare subclones, and the ability to monitor clonal change over time. Emerging technologies, such as single-cell sequencing, cell-free DNA assays, multi-omic profiling, and AI-driven analytics, are poised to enhance MRD monitoring and incorporate dynamic molecular data into precision-guided therapy.

Notwithstanding these advancements, obstacles persist, including assay standardization, identification of clinically meaningful thresholds, harmonization of reporting frameworks, and incorporation into regular clinical processes. Future clinical trials and consensus recommendations will be crucial to fully harness the potential of NGS MRD.

In summary, NGS-based MRD evaluation is set to become fundamental in AML management, facilitating individualized, risk-adjusted approaches that refine relapse prediction, direct focused interventions, and ultimately improve patient outcomes. Its incorporation into regular practice signifies a pivotal advancement toward precision treatment in AML. As these technologies evolve, comprehensive clinical validation, harmonized analytical criteria, and standardized reporting frameworks will be necessary to support their transition from high-resolution research tools to routine components of MRD-guided AML therapy.

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Original Article

Diagnosis and Treatment of HHV-8–Associated Multicentric Castleman Disease: a Clinical Case From the National Scientific Oncology Center

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Abstract:
Castleman disease (CD) is a rare lymphoproliferative disorder with heterogeneous clinical manifestations. This article presents a clinical case of HHV-8–associated multicentric Castleman disease (MCD) in an HIV-negative patient treated at the National Scientific Oncology Center. Diagnostic methods included histological and immunohistochemical evaluation, laboratory investigations, and imaging studies. The choice of therapy is discussed. The patient demonstrated resistance to rituximab monotherapy but achieved a favorable response to R-CHOP chemotherapy. This case highlights the importance of early assessment of HHV-8 status and an individualized approach to treatment selection.

Keywords: Castleman's Disease; Classification; Diagnosis; Treatment

Introduction

Castleman disease was first described by B. Castleman in 1954. Its morphological basis includes hyperplasia of lymphoid follicles with involution and hyalinization of germinal centers, along with prominent capillary proliferation in interfollicular zones featuring hyalinized vessel walls. Some vessels penetrate the hyperplastic follicles, producing the characteristic "lollipop" appearance.[2]

Variants of Castleman Disease

1. **Unicentric CD (UCD)** — affects a single lymph node or a single group. Symptoms may be absent or include lymphadenopathy, fatigue, fever, night sweats, and weight loss. Treatment typically involves surgical excision.
2. **Multicentric CD (MCD)** — involves multiple lymph node regions throughout the body and is associated with systemic symptoms such as fever, night sweats, weight loss, fatigue, anemia, and splenomegaly.

   MCD may be associated with human herpesvirus-8 (HHV-8), particularly in immunocompromised and HIV-positive patients.[3]

Histological Types

- **Hyaline-vascular type** — characterized by hyalinized germinal centers and vascular proliferation.
- **Plasma cell type** — prominent plasma cell infiltration, more common in MCD.
- **Mixed type.**

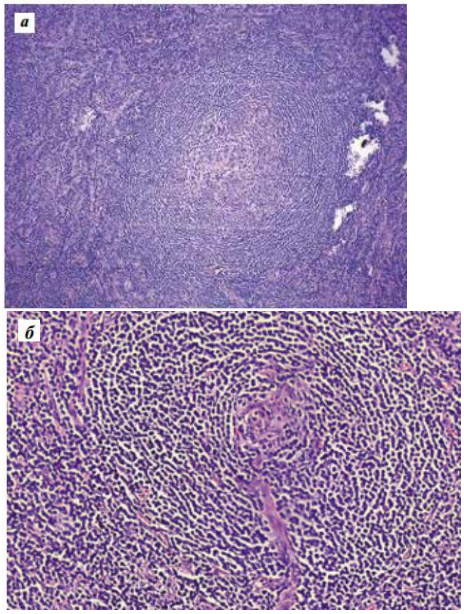

Fig. 1. Lymph node, hyaline-vascular variant:

a – regressed follicle with concentric mantle zones ("onion skin" appearance);

b – regressed follicle with hyalinized vessel penetrating the germinal center ("lollipop" sign). H&E ×100. [11]

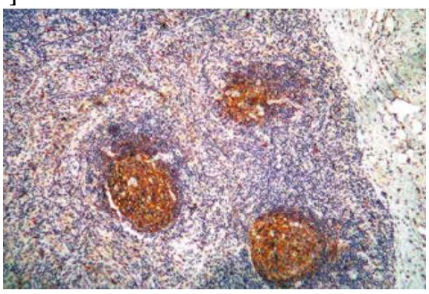

Fig. 2. Lymph node, plasma-cell variant. CD20 expression in hyperplastic follicles. IHC ×100. [11]

Clinical Features

- CD may mimic lymphoproliferative disorders such as lymphoma.
- Diagnosis relies on excisional biopsy, revealing characteristic features (follicular hyperplasia, angiogenesis, fibrosis).

According to NCCN guidelines, diagnosis requires differentiation of CD subtypes and exclusion of lymphoma and autoimmune diseases. Key steps include clinical assessment, laboratory testing, and lymph node biopsy.[7]

Essential Diagnostic Components

- Immunoglobulins: hypergammaglobulinemia (especially in MCD).
- IL-6: frequently elevated in CD, particularly in MCD.
- Viral testing:
- HHV-8 (strongly associated with MCD),
- HIV (HIV-positive individuals have higher risk).
- CT scan: evaluates lymphadenopathy and organ involvement.
- PET-CT: assesses active nodal disease and excludes malignancy.
- Ultrasound: evaluation of spleen, liver, large lymph nodes.
- Excisional lymph node biopsy — the diagnostic gold standard.

Histological Features

- UCD: follicular hyperplasia, vascular proliferation, hyalinization.
- MCD: more prominent vascularity, plasma cell infiltration.

Castleman disease is rare, with an estimated incidence of 21–25 cases per million per year.[5] It can occur at any age but is more common between ages 30–60. MCD is more prevalent in older adults; UCD can occur at any age. Some studies show a higher

incidence in males, particularly HHV-8–associated MCD.[5]

Epidemiology

- UCD: 70–80% of all CD cases
- MCD: 20–30% of cases, characterized by more severe systemic features.[6]

Etiology and Pathogenesis

Key factors include:

- Immune dysregulation: activation of T-cells and cytokine overproduction (notably IL-6).
- Viral infection: HHV-8 plays a major role in many MCD cases, particularly in immunocompromised hosts.[3]
- Genetic predisposition: reported in some cases.

Pathogenesis involves lymph node hyperplasia with abnormal follicular growth driven by IL-6, resulting in systemic manifestations such as fever and night sweats.[4]

Clinical Case

Patient O., 52 years old

Clinical Presentation and History

In April 2022, the patient developed paroxysmal dry cough, often accompanied by vomiting, marked weakness, dizziness, night sweats, and intensely pruritic skin eruptions. Periodic exacerbations lasted ~10 days with remissions of ~20 days. Pulmonary and infectious evaluations ruled out helminthiasis. Cetirizine partially relieved pruritus.

In 2023, the patient noted symmetrical enlargement of peripheral lymph nodes (cervical, axillary, inguinal), prompting further diagnostic workup.

Diagnostics

- Ultrasound and CT (2023): generalized lymphadenopathy; suspected lymphoproliferative disease.
- Fine needle aspiration / initial histology: suspicion of small B-cell lymphoma.
- Bone marrow exam: no evidence of lymphoma.
- Myelogram (Jan 2024): blasts 2.2%; hematopoiesis preserved; normal maturation.
- Bone marrow histology: reticulin fibrosis 0, no osteosclerosis, CD34+ ~3%, predominance of CD3/CD5+ T-cells, fewer CD20+ B-cells, no lymphoma.

Cervical lymph node excisional biopsy (April 2023)

- **Histology**: features typical of hyaline-vascular CD — hyalinized germinal centers, penetrating vessels, "onion-skin" mantle zones, marked vascular proliferation, increased plasma cells, compressed sinuses.
- **IHC:** normal expression of CD20, CD3, CD5, CD7; CD23+ follicular dendritic cells; weak CD10; Bcl-2 negative in germinal centers; Cyclin D1 negative; HHV-8–positive cells identified.

Conclusion: Castleman disease, hyaline-vascular type, HHV-8–associated, consistent with multicentric clinical presentation.

Clinical data at admission (April 2024)

Moderate anemia (Hb 108 g/L), elevated CRP (29.6 mg/L), generalized lymphadenopathy on CT.

HHV-8 PCR — negative (does not exclude local tissue infection).

HIV ELISA — negative.

Serum and urine immunofixation — no pathological findings.

Treatment

1. Rituximab monotherapy

After four infusions, disease progression was documented based on:

- persistent B-symptoms,
- PET-CT (15 July 2024): metabolically active cervical, supraclavicular, thoracic, axillary, retroperitoneal, pelvic, and inguinal lymph nodes; metabolic activity in Waldeyer's ring; enlarged hypermetabolic spleen; inflammatory activity in prostate; left-sided sinusitis.

According to NCCN Guidelines Version 1.2024 (Castleman Disease), treatment with siltuximab is recommended; however, due to unavailability, a decision was made to initiate R-CHOP chemotherapy.

2. Transition to R-CHOP (siltuximab unavailable)

- Cycle 1 (July 2024): improvement of B-symptoms.
- Cycle 2 (August 2024): well tolerated.

Response Evaluation

PET-CT (04 September 2024):

- SUVmax reduced from 3–9 to 0.7–2.6
- Reduction in lymph node size
- Normalization of spleen metabolism
- No abnormal uptake in internal organs

Conclusion: Complete metabolic response (Lugano).

Discussion

This case highlights the importance of comprehensive diagnostic evaluation, including viral studies, histopathology, and advanced imaging.

Features of HHV-8–associated MCD

- robust IL-6–mediated inflammation,
- potential resistance to rituximab,
- need for timely treatment escalation.

Current Therapeutic Approaches

- Rituximab — first-line therapy, but less effective in HHV-8–positive MCD.
- R-CHOP — effective in resistant or aggressive disease.
- Anti-IL-6 therapy (siltuximab) — mainly used in idiopathic MCD.
- Antiviral therapy — investigational.

Limitations of This Case

- Follow-up <24 months.
- Inability to administer anti-IL-6 therapy due to resource limitations.

Conclusion

This clinical case demonstrates key aspects of diagnosing and managing HHV-8–associated multicentric Castleman disease. Rituximab resistance and marked systemic inflammation required escalation to R-CHOP therapy, which resulted in a pronounced clinical and metabolic response. Comprehensive diagnosis, including HHV-8 tissue testing, is critical for appropriate treatment selection. Further research is needed to optimize management strategies for virus-associated forms of MCD.

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